Document made available under the Patent Cooperation Treaty (PCT)

International application number: PCT/US05/018412

International filing date: 23 May 2005 (23.05.2005)

Document type: Certified copy of priority document

Document details: Country/Office: US
Number: 60/633,013
Filing date: 03 December 2004 (03.12.2004)

Date of receipt at the International Bureau: 11 July 2005 (11.07.2005)

Remark: Priority document submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b)

World Intellectual Property Organization (WIPO) - Geneva, Switzerland
Organisation Mondiale de la Propriété Intellectuelle (OMPI) - Genève, Suisse

1339494

THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME:

UNITED STATES DEPARTMENT OF COMMERCE

United States Patent and Trademark Office

June 29, 2005

THIS IS TO CERTIFY THAT ANNEXED HERETO IS A TRUE COPY FROM THE RECORDS OF THE UNITED STATES PATENT AND TRADEMARK OFFICE OF THOSE PAPERS OF THE BELOW IDENTIFIED PATENT APPLICATION THAT MET THE REQUIREMENTS TO BE GRANTED A FILING DATE.

APPLICATION NUMBER: *60/633,013*
FILING DATE: *December 03, 2004*
RELATED PCT APPLICATION NUMBER: *PCT/US05/18412*

Certified by

Jon W. Dudas

Under Secretary of Commerce
for Intellectual Property
and Director of the United States
Patent and Trademark Office

U.S. DEPARTMENT OF COMMERCE
PATENT AND TRADEMARK OFFICE

PROVISIONAL APPLICATION FOR PATENT COVER SHEET	ATTORNEY DOCKET NO.: 395/44

Address to:
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

Inventor(s) and Residence(s) (city and either state or foreign country):

Gerard M. HOUSEY; Southfield, MI

For: **COMPOUNDS FOR OVERCOMING MUTATION-MEDIATED DRUG RESISTANCE**

1. 56 sheets of specification

2. 3 sheets of drawings.

3. 0 sheets of abstract

4. Please charge the required application filing fee of **$160.00 (large entity)**, and any other fees that may be required, to the deposit account of **Kenyon & Kenyon**, deposit account number **11-0600**. A duplicate of this sheet is enclosed.

5. Please direct all communications relating to this application to:

Lawrence P. Casson, Esq.
KENYON & KENYON
One Broadway
New York, New York 10004
(212) 425-7200 (phone)
(212) 425-5288 (facsimile)
CUSTOMER NUMBER 26646

6. This invention was not made by an agency of the United States Government or under a contract with an agency of the United States Government.

Respectfully submitted,

Dated: December 3, 2004

By: _____
Lawrence P. Casson, Reg. No. 46,606
KENYON & KENYON
One Broadway
New York, New York 10004
(212) 425-7200 (phone)
(212) 425-5288 (facsimile)
CUSTOMER NUMBER 26646

Express Mail No: EV 332462890 US

COMPOUNDS FOR OVERCOMING MUTATION-MEDIATED DRUG RESISTANCE

BACKGROUND OF THE INVENTION

[0001] The progressive development of drug resistance in a patient is the hallmark of chronic treatment with many classes of drugs, especially in the therapeutic areas of cancer and infectious diseases. Molecular mechanisms have been identified which mediate certain types of drug resistance phenomena, whereas in other cases the mechanisms of acquired as well as *de novo* resistance remain unknown today.

[0002] One mechanism of induced (acquired) drug resistance originally thought to be relevant in the area of cancer therapy involves increased expression of a protein known as P-glycoprotein (P-gp). P-gp is located in the cell membrane and functions as a drug efflux pump. The protein is capable of pumping toxic chemical agents, including many classical anti-cancer drugs, out of the cell. Consequently, upregulation of P-glycoprotein usually results in resistance to multiple drugs. Upregulation of P-glycoprotein in tumor cells may represent a defense mechanism which has evolved in mammalian cells to prevent damage from toxic chemical agents. Other related drug resistance proteins have now been identified with similar functions to P-gp, including multidrug-resistance-associated protein family members such as MRP1 and ABCG2. In any event, with the advent of the development of compounds that are specific for a given target protein, and less toxic, the importance of P-glycoprotein and related ATP-binding cassette (ABC) transporter proteins in clinically significant drug resistance has lessened.

[0003] Another possible molecular mechanism of acquired drug resistance is that alternative signal pathways are responsible for continued survival and metabolism of cells, even though the original drug is still effective against its target. Furthermore, alterations in intracellular metabolism of the drug can lead to loss of therapeutic efficacy as well. In addition, changes in gene expression as well as gene amplification events can occur, resulting in increased or decreased expression of a given target protein, and frequently requiring increasing dosages of the drug to maintain the same effects. (Adcock and Lane, 2003)

[0004] Mutation induced drug resistance is a frequently occurring event in the infectious disease area. For example, several drugs have been developed that inhibit either the viral reverse transcriptase or the viral protease encoded in the human immunodeficiency (HIV) viral genome. It is well established in the literature that repeated treatment of HIV-

infected AIDS patients using, for example, a reverse transcriptase inhibitor eventually gives rise to mutant forms of the virus that have reduced sensitivity to the drug which resulted from mutations that have occurred in the gene encoding reverse transcriptase that render the mutant form of the enzyme less affected by the drug.

[0005] The appearance of drug resistance during the course of HIV treatment is not surprising considering the rate at which errors are introduced into the HIV genome. The HIV reverse transcriptase enzyme is known to be particularly error prone, with a forward mutation rate of about 3.4×10^{-5} mutations per base pair per replication cycle (Mansky et al., *J. Virol.* 69:5087-94 (1995)). However, analogous mutation rates for endogenous genes encoded in mammalian cells are more than an order of magnitude lower.

[0006] New evidence shows that drug resistance can also arise from a mutational event involving the gene encoding the drug target (Gorre et al., Science, 2001; PCT/US/02/18729). In this case, exposure of the patient to a specific therapeutic substance such as a given cancer drug that targets a specific ***protein-of-interest*** (POI, or "target" protein) may be followed by the outgrowth of a group of cells harboring a mutation occurring in the gene encoding the protein that is the target of the therapeutic substance. Whether the outgrowth of this population of cells results from a small percentage of pre-existing cells in the patient which already harbor a mutation which gives rise to a drug-resistant POI, or whether such mutations arise *de novo* during or following exposure of the animal or human being to a therapeutic agent capable of activating or inhibiting said POI, is presently unknown. In either case, such mutation events may result in a mutated protein (hereinafter defined as a ***theramutein***) which is less affected, or perhaps completely unaffected, by said therapeutic substance.

[0007] Chronic myologenous leukemia (CML) is characterized by excess proliferation of myeloid progenitors that retain the capacity for differentiation during the stable or chronic phase of the disease. Multiple lines of evidence have established deregulation of the Abl tyrosine kinase as the causative oncogene in certain forms of CML. The deregulation is commonly associated with a chromosomal translocation known as the Philadelphia chromosome, which results in expression of a fusion protein, $p210^{Bcr\text{-}Abl}$, which has tyrosine kinase activity. Transformation appears to result from activation of multiple signal pathways including those involving RAS, MYC, and JUN. Imatinib mesylate (also termed STI-571, "Gleevec") is a 2-phenylamino pyrimidine that targets the ATP binding site

of the kinase domain of Abl (Drucker et al, NEJM 2001, p. 1038). Subsequently it has also been found by other methods to be an inhibitor of platelet-derived growth factor β receptor, and the Kit tyrosine kinase, the latter of which is involved in the development of gastrointestinal stromal tumors (see below).

[0008] Until recently, it had not been observed that during the course of treatment with a specific inhibitor of a given endogenous cellular protein that a mutation in its corresponding *endogenous* gene could lead to the expression of protein variants whose cellular functioning was resistant to the inhibitor. Work by Charles Sawyers and colleagues (Gorre et al., Science 293:876-80 (2001); see also PCT/US/02/18729) demonstrated for the first time that treatment of a patient with a drug capable of inhibiting the $p210^{Bcr\text{-}Abl}$ tyrosine kinase, i.e. STI-571 (imatinib mesylate, or "Gleevec®") could be followed by the emergence of a clinically significant population of cells within said patient harboring a mutation in the gene encoding the $p210^{Bcr\text{-}Abl}$ cancer causing target protein which contains the Abelson tyrosine kinase domain. Various such mutations gave rise to mutant forms of $p210^{Bcr\text{-}Abl}$ which were less responsive to Gleevec treatment than was the original cancer causing version. Notably, the mutations that emerged conferred upon the mutant protein a relative resistance to the effects of the protein kinase inhibitor drug, while maintaining a certain degree of the original substrate specificity of the mutant protein kinase. Prior to Gorre et al.'s work, it was generally believed by those skilled in the art that the types of resistance that would be observed in patients exposed to a compound which inhibited the Abelson protein kinase, such as STI-571, would have resulted from one or more of the other mechanisms of drug resistance listed above, or by some other as yet unknown mechanism, but that in any event said resistance would involve a target (protein or otherwise) which was distinct from the drug's target POI.

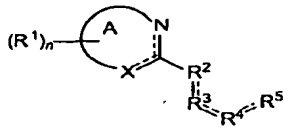
[0009] Accordingly, the ability to treat clinically relevant resistant mutant forms of proteins that are otherwise the targets of an existing therapy would be extremely useful. Such mutated proteins (theramuteins) are beginning to be recognized and understood to be important targets in recurring cancers, and will become important in other diseases as well. There exists a need for therapeutic agents that are active against such drug resistant variant forms of cellular proteins that may arise before, during or following normally effective drug therapies. A key purpose of this invention is to provide compounds that may serve as

potential therapeutic agents useful in overcoming mutation-induced drug resistance in endogenously occurring proteins.

BRIEF SUMMARY OF THE INVENTION

[0010] This invention relates to agents that are inhibitors or activators of variant forms of endogenous proteins. Of particular interest are inhibitors and activators of endogenous protein variants, encoded by genes which have mutated, and which often arise or are at least first identified as having arisen following exposure to a chemical agent which is known to be an inhibitor or activator of the corresponding unmutated endogenous protein. Such mutant proteins, herein termed "theramuteins," may occur either spontaneously in an organism (and be pre-existing mutations in some cases), or said mutants may arise as a result of selective pressure which results when the organism is treated with a given chemical agent capable of inhibiting the non-mutated form of said theramutein (herein termed a "prototheramutein"). It will be understood that in some cases a prototheramutein may be a "wild type" form of a POI (*e.g.*, a protein that gives rise to a disease due to disregulation). In other cases, the prototheramutein will be a disease causing variant of a "wild type" protein, having already mutated and thereby contributing to the development of the diseased state as a result of said prior mutation. One example of the latter type of prototheramutein is the $P210^{BCR\text{-}ABL}$ oncoprotein, and a mutant form of this protein harboring a threonine (T) to isoleucine (I) mutation at position 315 is termed $P210^{BCR\text{-}ABL\text{-}T315I}$ and is one example of a theramutein.

[0011] In a preferred embodiment, the present invention provides inhibitors of the $P210^{BCR\text{-}ABL\text{-}T315I}$ theramutein having the formula I



(I)

wherein:

ring A is a 5-, 6-, or 7- membered heterocyclic ring or a 7- to 12-membered fused heterobicyclic ring;

X is N or C-R^1;

the dotted lines represent optional double bonds;

each R^1 is independently selected from the group consisting of H, alkyl, cycloalkyl, alkenyl,

alkynyl, aralkyl, CN, CF_3, NR_2, NO_2, OR, CO_2R, C(O)R, halo, aryl, and a heterocyclic ring, and additionally or alternatively, two R^1 groups on adjacent ring atoms form a 5- or 6-membered fused ring which contains from 0 to 3 heteroatoms;

n is 0 to 6,

R^2 is selected from $-CR^{11}{}_a-$ and $-NR^{12}{}_b-$;

each R^{11} is independently selected from H, halo, $-NH_2$, $-N(H)(C_{1-3}$ alkyl), $-N(C_{1-3}$ alkyl$)_2$, $-O-(C_{1-3}$ alkyl), OH and C_{1-3} alkyl;

each R^{12} is independently selected from H and C_{1-3} alkyl;

R^3 is selected from $-CR^{13}{}_c-$, $-NR^{14}{}_d-$, and $-(C=R^{17})-$;

each R^{13} group is selected from H, halo, $-NH_2$, -N(H)(R), $-NR_2$, -O-R, OH and C_{1-3} alkyl

each R^{14} group is selected from H, alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, CO_2R, C(O)R, aryl, and a heterocyclic ring;

R^4 is selected from $-CR^{15}{}_e-$, $-NR^{16}{}_f-$, $-(C=R^{17})-$, and -O-;

each R^{15} is selected from H, alkyl, cycloalkyl, alkenyl, alkynyl, CO_2R, C(O)R, aralkyl, aryl, and a heterocyclic ring;

each R^{16} group is selected from H, alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, CO_2R, C(O)R, aryl, and a heterocyclic ring;

each R^{17} is selected from O, S, N-R, and N-OR;

with the provisos that when R^2 is $-NR^{12}{}_b-$ and R^4 is $-NR^{16}{}_f-$, then R^3 is not $-NR^{14}{}_d-$; and that both R^3 and R^4 are not simultaneously selected from $-(C=R^{17})-$;

R^5 is selected from $-Y-R^6$ and $-Z-R^7$;

Y is selected from a chemical bond, O, N-R,

R^6 is selected from alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, aryl, and a heterocyclic ring;

Z is a hydrocarbon chain having from 1 to 4 carbon atoms, and optionally substituted with one or more of halo, alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, CO_2R, C(O)R, CN, CF_3, NR_2, NO_2, and OR;

R^7 is H or is selected from aryl and a heterocyclic ring;

each R is independently selected from H, alkyl, cycloalkyl, aralkyl, aryl and a heterocyclic ring;

a is 1 or 2;

b is 0 or 1;

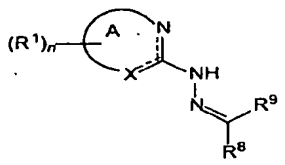
c is 1 or 2;

d is 0 or 1;

e is 1 or 2; and

f is 0 or 1.

[0012] In a further preferred embodiment, the present invention provides inhibitors of the $P210^{BCR\text{-}ABL\text{-}T315I}$ theramutein having the formula II



(II)

wherein

X is N or C-R^1;

ring A is a 5-, 6-, or 7- membered heterocyclic ring or a 7- to 12-membered fused heterobicyclic ring;

the dotted lines represent optional double bonds,

each R^1 is independently selected from the group consisting of alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, CN, CF_3, NR_2, NO_2, OR, CO_2R, C(O)R, halo, aryl, and a heterocyclic ring, and additionally or alternatively, two R^1 groups on adjacent ring atoms form a fused 5- or 6-membered fused ring which contains from 0 to 3 heteroatoms;

n is 0 to 6,

R^8 is selected from the group consisting of H, alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl aryl, and a heterocyclic ring;

R^9 is selected from -Y-R^6 and -Z-R^7;

Y is selected from a chemical bond, O, N-R,

R^6 is selected from alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, aryl, and a heterocyclic ring;

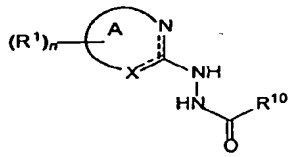
Z is a hydrocarbon chain having from 1 to 4 carbon atoms, and optionally substituted with one or more of halo, alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, CO_2R, C(O)R, CN, CF_3, NR_2, NO_2, and OR;

R^7 is H or is selected from aryl and a heterocyclic ring; and

each R is independently selected from H, alkyl, cycloalkyl, aralkyl, aryl and a heterocyclic ring; and

n is 0 to 6.

[0013] In a further preferred embodiment, the present invention provides inhibitors of the $P210^{BCR\text{-}ABL\text{-}T315I}$ theramutein having the formula III



(III)

wherein

X is N or C-R^1;

ring A is a 5-, 6-, or 7- membered heterocyclic ring or a 7- to 12-membered fused heterobicyclic ring;

the dotted lines represent optional double bonds;

each R^1 is independently selected from the group consisting of alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, CN, CF_3, NR_2, NO_2, OR, CO_2R, C(O)R, halo, aryl, and a heterocyclic ring, and additionally or alternatively, two R^1 groups on adjacent ring atoms form a fused 5- or 6-membered ring which contains from 0 to 3 heteroatoms;

R^{10} is selected from -Y'-R^{18};

Y' is selected from a chemical bond, O, NR-, and a hydrocarbon chain having from 1 to 4 carbon atoms, and optionally substituted with one or more of halo, alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, CO_2R, C(O)R, CN, CF_3, NR_2, NO_2, and OR;

R^{18} is selected from the group consisting of H, alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, CF_3, aryl, and a heterocyclic ring;

each R is independently selected from H, alkyl, cycloalkyl, aralkyl, aryl and a heterocyclic ring; and

n is 0 to 6.

[0014] The invention provides for a fundamentally new way of treating cancer and other diseases where treatment with an existing drug compound, by whatever mechanism, is followed by identifiable (clinically significant) theramutein-mediated drug resistance, by providing alternative drugs that can be administered as theramuteins arise and are identified

as such (Wakai et al.; 2004 reports an example wherein a theramutein may arise during the course of an on-going treatment regimen), or preemptively before the outgrowth of clinically significant populations of theramutein expressing cells. Further, where a drug treatment for a particular disease is less effective in a subset of individuals that express a certain theramutein of a protein that the drug targets, the invention enables the tailoring of treatments provided to those subjects by providing alternative drug substances.

DESCRIPTION OF THE FIGURES

[0015] Figure 1 shows the effect on growth and viability of different concentrations of Compound 2 (C2) for non-transformed vector control Ba/F3 cells (which are IL-3 dependent) as well as Ba/F3 cells expressing the "wild type" $p210^{Bcr\text{-}Abl}$ (designated $p210^{Bcr\text{-}Abl\text{-}wt}$), and Ba/F3 cells expressing the $p210^{Bcr\text{-}Abl\text{-}T315I}$ drug resistant mutant. Cell counts and viability were determined on an automated cell counter as discussed in detail in the specification. Cell counts are shown by the solid color bars; cell viability is shown by the hashed bars. Note that STI-571 potently inhibits growth of the P210 cell line (grey bar) whereas it is unable to inhibit the growth of the T315I cell line (white bar) even at 10 μM concentration. 500 nM C2 shows the largest specificity gap within this dose-response series. Compare STI-571 at 10 μM to C2 at 500 nM on the T315I cell line (white bars). Abbreviations: DMSO: dimethylsulfoxide (solvent used for drug dissolution).

[0016] Figure 2 shows various determinations of the specificity gap by comparing the effect of STI-571 and Test Compound 2 (C2) identified in the screen in terms of their effects on the prototheramutein- and theramutein-expressing cell lines. Panel A: control DMSO treatments; B: negative heterologous specificity gap; C: slightly positive heterologous specificity gap; D: large positive homologous specificity gap; See text for explanations.

[0017] Figure 3 shows an autoradiograph of recombinant P210 Bcr-Abl wild type and T315I mutant kinase domains assayed for autophosphorylation activity. 200 ng of protein were preincubated with test substances for 10 minutes under standard autophosphoryation reaction conditions and then radiolabelled ATP was added and the reactions proceeded for 30 minutes at 30°C, after which the samples were separated by SDS-PAGE. The gels were silver-stained, dried down under vacuum and exposed to X-ray film. Note that whereas 10 μM STI 571 is effective against wild type P210 Bcr-Abl, it is virtually ineffective against the T315I kinase domain, even at concentrations up to 100 μM.

DETAILED DESCRIPTION OF THE INVENTION

[0018] The term "halo" or "halogen" as used herein includes fluorine, chlorine, bromine and iodine.

[0019] The term "alkyl" as used herein contemplates both straight and branched chain alkyl radicals having from 1 to 6 carbon atoms. Preferred alkyl groups include methyl, ethyl, propyl, isopropyl, butyl, isobutyl, *tert*-butyl, and the like. Additionally, the alkyl group may bc optionally substituted with one or more substituents selected from halo, CN, CO_2R, C(O)R, NR_2, cyclic-amino, NO_2, and OR, in which R is selected from H, alkyl, cycloalkyl, aralkyl, aryl and a heterocyclic ring..

[0020] The term "cycloalkyl" as used herein contemplates cyclic alkyl radicals. Preferred cycloalkyl groups are those containing 3 to 7 carbon atoms and includes cyclopropyl, cyclopentyl, cyclohexyl, and the like. Additionally, the cycloalkyl group may be optionally substituted with one or more substituents selected from halo, CN, CO_2R, C(O)R, NR_2, cyclic-amino, NO_2, and OR.

[0021] The term "alkenyl" as used herein contemplates both straight and branched chain alkene radicals. Preferred alkenyl groups are those containing two to six carbon atoms. Additionally, the alkenyl group may be optionally substituted with one or more substituents selected from halo, CN, CO_2R, C(O)R, NR_2, cyclic-amino, NO_2, and OR.

[0022] The term "alkynyl" as used herein contemplates both straight and branched chain alkyne radicals. Preferred alkynyl groups are those containing two to six carbon atoms. Additionally, the alkynyl group may be optionally substituted with one or more substituents selected from halo, CN, CO_2R, C(O)R, NR_2, cyclic-amino, NO_2, and OR.

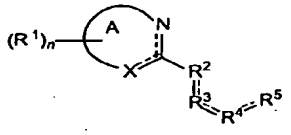
[0023] The term "aralkyl" as used herein contemplates an alkyl group which has as a substituent an aromatic group. Additionally, the aralkyl group may be optionally substituted on the aryl with one or more substituents selected from halo, alkyl, alkenyl, CN, CO_2R, C(O)R, NR_2, cyclic-amino, NO_2, and OR.

[0024] The term "heterocyclic group" or "heterocyclic ring" as used herein contemplates aromatic and non-aromatic cyclic radicals having at least one heteroatom as a ring member. Preferred heterocyclic groups are those containing 5 or 6 ring atoms which includes at least one hetero atom, and includes cyclic amines such as morpholino, piperidino, pyrrolidino, and the like, and cyclic ethers, such as tetrahydrofuran, tetrahydropyran, and the like. Aromatic heterocyclic groups, also termed "heteroaryl" groups contemplates single-ring

hetero-aromatic groups that may include from one to three heteroatoms, for example, pyrrole, furan, thiophene, imidazole, oxazole, thiazole, triazole, pyrazole, pyridine, pyrazine, pyridazine, pyrimidine, and the like. The term heteroaryl also includes polycyclic hetero-aromatic systems having two or more rings in which two atoms are common to two adjoining rings (the rings are "fused") wherein at least one of the rings is a heteroaryl, e.g., the other rings can be cycloalkyls, cycloalkenyls, aryl, heterocycles and/or heteroaryls. Examples of polycyclic heteroaromatic systems include quinoline, isoquinoline, tetrahydroisoquinoline, quinoxaline, quinaxoline, benzimidazole, benzofuran, purine, imidazopyridine, benzotriazole, and the like. Additionally, the heterocyclic groups may be optionally substituted with one or more substituents selected from halo, alkyl, aralkyl, alkenyl, CN, CO_2R, C(O)R, NR_2, cyclic-amino, NO_2, and OR.

[0025] The term "aryl" or "aromatic group" as used herein contemplates single-ring aromatic groups (for example, phenyl, pyridyl, pyrazole, etc.) and polycyclic ring systems (naphthyl, quinoline, etc.). The polycyclic rings may have two or more rings in which two atoms are common to two adjoining rings (the rings are "fused") wherein at least one of the rings is aromatic, e.g., the other rings can be cycloalkyls, cycloalkenyls, aryl, heterocycles and/or heteroaryls. Additionally, the aryl groups may be optionally substituted with one or more substituents selected from halo, alkyl, aralkyl, alkenyl, CN, CO_2R, C(O)R, NR_2, cyclic-amino, NO_2, OR, alkynyl, CF_3,NH(C=O)R, NH(C=O)NHR, or $NHSO_2R$.

[0026] In a preferred embodiment, the present invention provides inhibitors of the $P210^{BCR\text{-}ABL\text{-}T315I}$ theramutein having the formula I



(I)

wherein:

ring A is a 5-, 6-, or 7- membered heterocyclic ring or a 7- to 12-membered fused heterobicyclic ring;

X is N or C-R^1;

the dotted lines represent optional double bonds;

each R^1 is independently selected from the group consisting of H, alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, CN, CF_3, NR_2, NO_2, OR, CO_2R, C(O)R, halo, aryl, and a heterocyclic ring, and additionally or alternatively, two R^1 groups on adjacent ring atoms form a 5- or 6-

membered fused ring which contains from 0 to 3 heteroatoms;

n is 0 to 6,

R^2 is selected from $-CR^{11}{}_a-$ and $-NR^{12}{}_b-$;

each R^{11} is independently selected from H, halo, $-NH_2$, $-N(H)(C_{1-3}$ alkyl), $-N(C_{1-3}$ alkyl$)_2$, $-O-(C_{1-3}$ alkyl), OH and C_{1-3} alkyl;

each R^{12} is independently selected from H and C_{1-3} alkyl;

R^3 is selected from $-CR^{13}{}_c-$, $-NR^{14}{}_d-$, and $-(C=R^{17})-$;

each R^{13} group is selected from H, halo, $-NH_2$, -N(H)(R), $-NR_2$, -O-R, OH and C_{1-3} alkyl

each R^{14} group is selected from H, alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, CO_2R, C(O)R, aryl, and a heterocyclic ring;

R^4 is selected from $-CR^{15}{}_e-$, $-NR^{16}{}_f-$, $-(C=R^{17})-$, and -O-;

each R^{15} is selected from H, alkyl, cycloalkyl, alkenyl, alkynyl, CO_2R, C(O)R, aralkyl, aryl, and a heterocyclic ring;

each R^{16} group is selected from H, alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, CO_2R, C(O)R, aryl, and a heterocyclic ring;

each R^{17} is selected from O, S, N-R, and N-OR;

with the provisos that when R^2 is $-NR^{12}{}_b-$ and R^4 is $-NR^{16}{}_f-$, then R^3 is not $-NR^{14}{}_d-$; and that both R^3 and R^4 are not simultaneously selected from $-(C=R^{17})-$;

R^5 is selected from $-Y-R^6$ and $-Z-R^7$;

Y is selected from a chemical bond, O, N-R,

R^6 is selected from alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, aryl, and a heterocyclic ring;

Z is a hydrocarbon chain having from 1 to 4 carbon atoms, and optionally substituted with one or more of halo, alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, CO_2R, C(O)R, CN, CF_3, NR_2, NO_2, and OR;

R^7 is H or is selected from aryl and a heterocyclic ring;

each R is independently selected from H, alkyl, cycloalkyl, aralkyl, aryl and a heterocyclic ring;

a is 1 or 2;

b is 0 or 1;

c is 1 or 2;

d is 0 or 1;

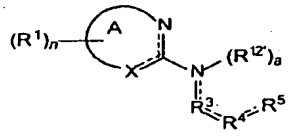
e is 1 or 2; and

f is 0 or 1.

[0027] In a preferred embodiment, if R^2 is selected to be $-NR^{12}{}_b-$, then R^{13} is not selected from halo, $-NH_2$, -N(H)(R), $-NR_2$, -O-R, or OH.

[0028] When one or more chiral centers are present in the compounds of the present invention, the individual isomers and mixtures thereof (*e.g.*, racemates, etc.) are intended to be encompassed by the formuale dipicted herein. Both the *cis* and *trans* isomers of compounds of the present invention are contemplated.

[0029] In a further preferred embodiment, the present invention provides inhibitors of the $P210^{BCR\text{-}ABL\text{-}T315I}$ theramutein having the formula I_a



(I_a)

wherein:

ring A is a 5-, 6-, or 7- membered heterocyclic ring or a 7- to 12-membered fused heterobicyclic ring;

X is N or C-R^1;

the dotted lines represent optional double bonds;

each R^1 is independently selected from the group consisting of H, alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, CN, CF_3, NR_2, NO_2, OR, CO_2R, C(O)R, halo, aryl, and a heterocyclic ring, and additionally or alternatively, two R^1 groups on adjacent ring atoms form a 5- or 6-membered fused ring which contains from 0 to 3 heteroatoms;

n is 0 to 6,

each R^{12} is independently selected from H and $C_{1\text{-}3}$ alkyl;

R^3 is selected from $-CR^{13}{}_c-$, $-NR^{14}{}_d-$, and $-(C=R^{17})-$;

each R^{13} group is selected from H, halo, $-NH_2$, -N(H)(R), $-NR_2$, -O-R, OH and $C_{1\text{-}3}$ alkyl

each R^{14} group is selected from H, alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, CO_2R, C(O)R, aryl, and a heterocyclic ring;

R^4 is selected from $-CR^{15}{}_e-$, $-NR^{16}{}_f-$, $-(C=R^{17})-$, and -O-;

each R^{15} is selected from H, alkyl, cycloalkyl, alkenyl, alkynyl, CO_2R, C(O)R, aralkyl, aryl, and a heterocyclic ring;

each R^{16} group is selected from H, alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, CO_2R, C(O)R, aryl, and a heterocyclic ring;

each R^{17} is selected from O, S, N-R, and N-OR;

with the provisos that when R^2 is $-NR^{12}{}_b-$ and R^4 is $-NR^{16}{}_f-$, then R^3 is not $-NR^{14}{}_d-$; and that both R^3 and R^4 are not simultaneously selected from $-(C=R^{17})-$;

R^5 is selected from $-Y-R^6$ and $-Z-R^7$;

Y is selected from a chemical bond, O, N-R,

R^6 is selected from alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, aryl, and a heterocyclic ring;

Z is a hydrocarbon chain having from 1 to 4 carbon atoms, and optionally substituted with one or more of halo, alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, CO_2R, C(O)R, CN, CF_3, NR_2, NO_2, and OR;

R^7 is H or is selected from aryl and a heterocyclic ring;

each R is independently selected from H, alkyl, cycloalkyl, aralkyl, aryl and a heterocyclic ring;

b is 0 or 1;

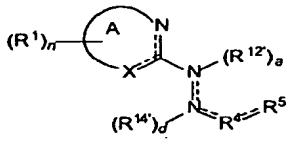
c is 1 or 2;

d is 0 or 1;

e is 1 or 2; and

f is 0 or 1.

[0030] In a further preferred embodiment, the present invention provides inhibitors of the $P210^{BCR\text{-}ABL\text{-}T315I}$ theramutein having the formula I_b



(I_b)

wherein:

ring A is a 5-, 6-, or 7- membered heterocyclic ring or a 7- to 12-membered fused heterobicyclic ring;

X is N or C-R^1;

the dotted lines represent optional double bonds;

each R^1 is independently selected from the group consisting of H, alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, CN, CF_3, NR_2, NO_2, OR, CO_2R, C(O)R, halo, aryl, and a heterocyclic ring, and additionally or alternatively, two R^1 groups on adjacent ring atoms form a 5- or 6-membered fused ring which contains from 0 to 3 heteroatoms;

n is 0 to 6,

each R^{12} is independently selected from H and C_{1-3} alkyl;

each R^{14} group is selected from H, alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, CO_2R, C(O)R, aryl, and a heterocyclic ring;

R^4 is selected from $-CR^{15}_{e}-$, $-NR^{16}_{f}-$, and -(C=R^{17})-, and -O-;

each R^{15} is selected from H, alkyl, cycloalkyl, alkenyl, alkynyl, CO_2R, C(O)R, aralkyl, aryl, and a heterocyclic ring;

each R^{16} group is selected from H, alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, CO_2R, C(O)R, aryl, and a heterocyclic ring;

each R^{17} is selected from O, S, N-R, and N-OR;

with the provisos that when R^2 is $-NR^{12}_{b}-$ and R^4 is $-NR^{16}_{f}-$, then R^3 is not $-NR^{14}_{d}-$; and that both R^3 and R^4 are not simultaneously selected from -(C=R^{17})-;

R^5 is selected from -Y-R^6 and -Z-R^7;

Y is selected from a chemical bond, O, N-R,

R^6 is selected from alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, aryl, and a heterocyclic ring;

Z is a hydrocarbon chain having from 1 to 4 carbon atoms, and optionally substituted with one or more of halo, alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, CO_2R, C(O)R, CN, CF_3, NR_2, NO_2, and OR;

R^7 is H or is selected from aryl and a heterocyclic ring;

each R is independently selected from H, alkyl, cycloalkyl, aralkyl, aryl and a heterocyclic ring;

b is 0 or 1;

d is 0 or 1;

e is 1 or 2; and

f is 0 or 1.

[0031] In preferred embodiments of the invention, R^2, R^3 and R^4 of formula I are selected to give the following chemical groups:

$-N(R^{12})-N=C(R^{15})-$

$-N(R^{12})-N(R^{14})-C(=O)-$

$-N(R^{12})-N(R^{14})-C(R^{15})(R^{15})-$

$-N(R^{12})-C(R^{13})(R^{13})-C(R^{15})(R^{15})-$

$-N(R^{12})-C(R^{13})(R^{13})-C(=O)-$

$-N=N-C(R^{15})(R^{15})-$

$-C(R^{11})=C=C(R^{15})-$

$-C(R^{11})=C(R^{13})-C(=O)-$

$-C(R^{11})=C(R^{13})-C(R^{15})(R^{15})-$

$-C(R^{11})(R^{11})-C(R13)=C(R^{15})-$

$-C(R^{11})(R^{11})-C(R^{13})(R^{13})-C(=O)-$

$-C(R^{11})(R^{11})-C(R^{13})(R^{13})-C(R^{15})(R^{15})-$

$-C(R^{11})(R^{11})-N(R^{14})-C(=O)-$

$-C(R^{11})(R^{11})-N(R^{14})-C(R^{15})(R^{15})-$

$-N(R^{12})-C(=O)-C(R^{15})(R^{15})-$

$-N(R^{12})-C(=O)-N(R^{16})-$

$-N(R^{12})-C(=O)-O-$

$-C(R^{11})(R^{11})-C(=O)-C(R^{15})(R^{15})-$

$-C(R^{11})(R^{11})-C(=O)-N(R^{16})-$.

Particularly preferred chemical groups for R^2, R^3 and R^4 include:

$-N(R^{12})-N=C(R^{15})-$

$-N(R^{12})-N(R^{14})-C(=O)-$

$-N(R^{12})-C(R^{13})(R^{13})-C(R^{15})(R^{15})-$

$-N(R^{12})-C(R^{13})(R^{13})-C(=O)-$

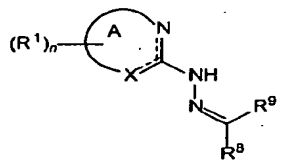
$-C(R^{11})(R^{11})-C(=O)-C(R^{15})(R^{15})-$

$-C(R^{11})(R^{11})-C(=O)-N(R^{16})-$.

[0032] In further preferred embodiment, R^6 or R^7 is an aryl group, which may be optionally substituted. Particularly preferred aryl groups include substituted or unsubstituted phenyl and pyridyl. In further preferred embodiments each R^8 is O. In additional or alternative embodiments, it is preferred that the substituents R^{11} and R^{12} are independently

selected from groups which have small steric bulk and are preferably selected from H, halo, OH, NH_3 and CH_3, and more preferably are H.

[0033] In a further preferred embodiment, the present invention provides inhibitors of the $P210^{BCR\text{-}ABL\text{-}T315I}$ theramutein having the formula II



(II)

wherein

X is N or C-R^1;

ring A is a 5-, 6-, or 7- membered heterocyclic ring or a 7- to 12-membered fused heterobicyclic ring;

the dotted lines represent optional double bonds,

each R^1 is independently selected from the group consisting of alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, CN, CF_3, NR_2, NO_2, OR, CO_2R, C(O)R, halo, aryl, and a heterocyclic ring, and additionally or alternatively, two R^1 groups on adjacent ring atoms form a fused 5- or 6-membered fused ring which contains from 0 to 3 heteroatoms;

n is 0 to 6,

R^8 is selected from the group consisting of H, alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl aryl, and a heterocyclic ring;

R^9 is selected from -Y-R^6 and -Z-R^7;

Y is selected from a chemical bond, O, N-R,

R^6 is selected from alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, aryl, and a heterocyclic ring;

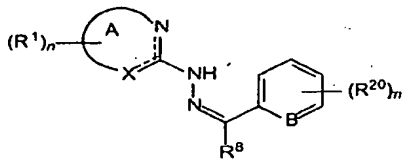
Z is a hydrocarbon chain having from 1 to 4 carbon atoms, and optionally substituted with one or more of halo, alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, CO_2R, C(O)R, CN, CF_3, NR_2, NO_2, and OR;

R^7 is H or is selected from aryl and a heterocyclic ring; and

each R is independently selected from H, alkyl, cycloalkyl, aralkyl, aryl and a heterocyclic ring; and

n is 0 to 6.

[0034] In a further preferred embodiment, the present invention provides inhibitors of the $P210^{BCR\text{-}ABL\text{-}T315I}$ theramutein having the formula II_a



(II_a)

wherein

X is N or C-R^1;

ring A is a 5-, 6-, or 7- membered heterocyclic ring or a 7- to 12-membered fused heterobicyclic ring;

the dotted lines represent optional double bonds,

each R^1 is independently selected from the group consisting of alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, CN, CF_3, NR_2, NO_2, OR, CO_2R, C(O)R, halo, aryl, and a heterocyclic ring, and additionally or alternatively, two R^1 groups on adjacent ring atoms form a fused 5- or 6-membered fused ring which contains from 0 to 3 heteroatoms;

n is 0 to 6,

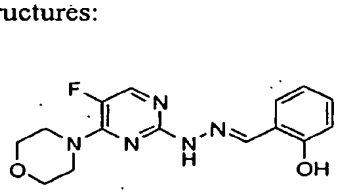
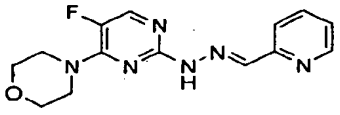
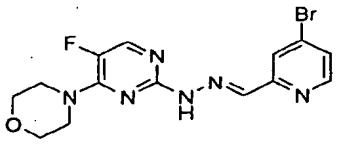
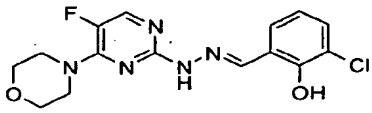
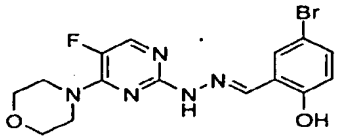
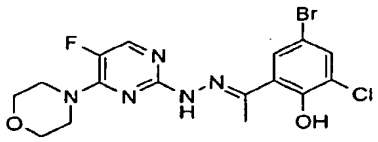
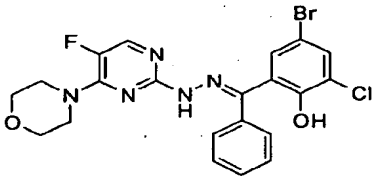
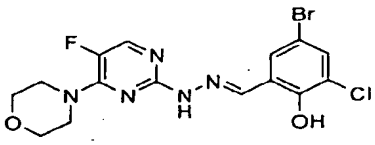
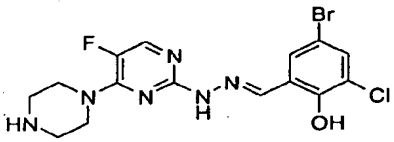
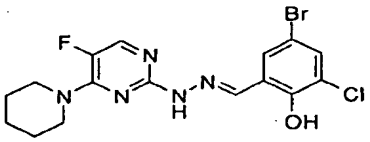
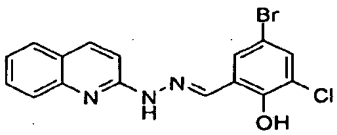
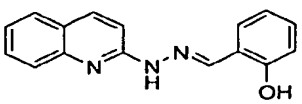
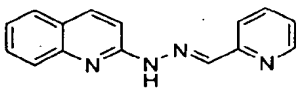
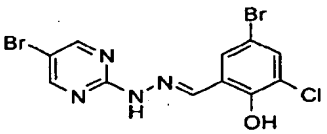
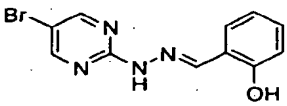
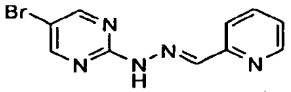
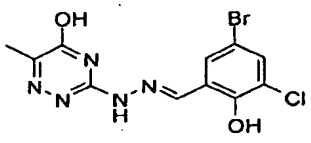
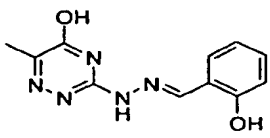
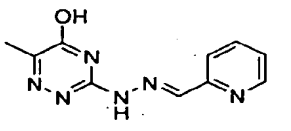
R^8 is selected from the group consisting of H, alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl aryl, and a heterocyclic ring;

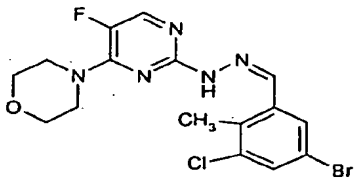
B is N, CH or C-R^{20};

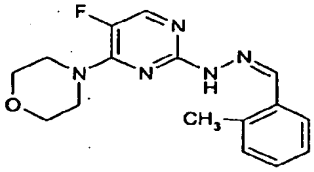
each R^{20} is independently selected from the group consisting of alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, CN, CF_3, NR_2, NO_2, OR, CO_2R, C(O)R, halo, aryl, and a heterocyclic ring, and additionally or alternatively, two R^{20} groups on adjacent ring atoms form a fused 5- or 6-membered fused ring which contains from 0 to 3 heteroatoms; and

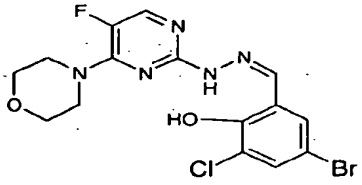
m is 0 to 6.

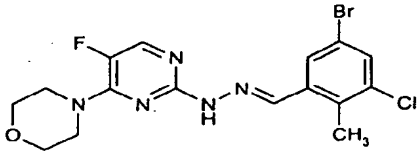
[0035] Exemplary compounds of the formula II or II_a includes the following structures:

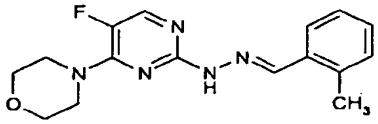




















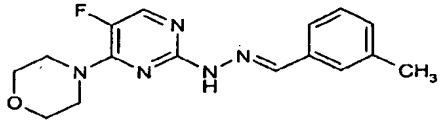


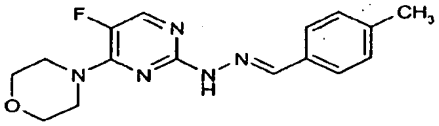


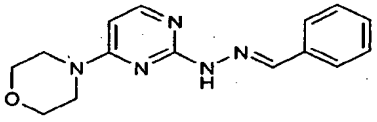


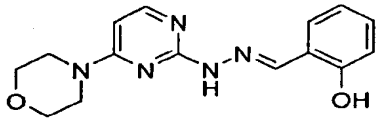


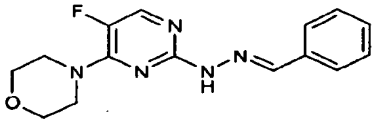




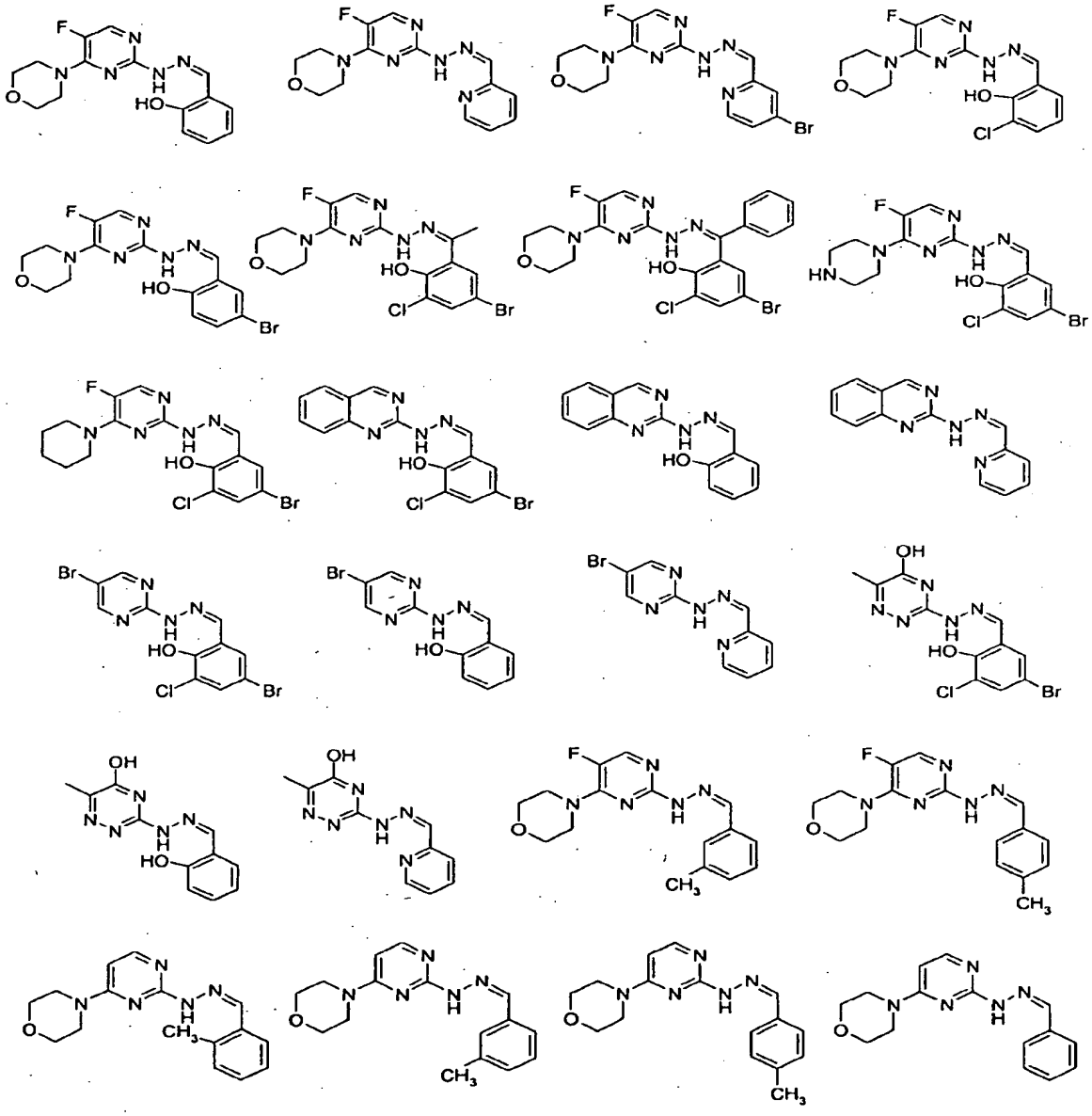




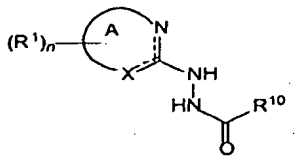




[0036] Additional exemplary compounds of the formula II or II_a include:



[0037] In a further preferred embodiment, the present invention provides inhibitors of the $P210^{BCR\text{-}ABL\text{-}T315I}$ theramutein having the formula III



(III)

wherein

X is N or C-R^1;

ring A is a 5-, 6-, or 7- membered heterocyclic ring or a 7- to 12-membered fused heterobicyclic ring;

the dotted lines represent optional double bonds;

each R^1 is independently selected from the group consisting of alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, CN, CF_3, NR_2, NO_2, OR, CO_2R, C(O)R, halo, aryl, and a heterocyclic ring, and additionally or alternatively, two R^1 groups on adjacent ring atoms form a fused 5- or 6-membered ring which contains from 0 to 3 heteroatoms;

R^{10} is selected from -Y'-R^{18};

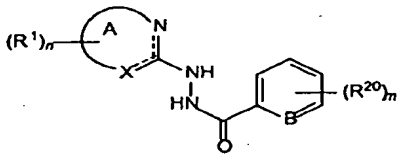
Y' is selected from a chemical bond, O, NR-, and a hydrocarbon chain having from 1 to 4 carbon atoms, and optionally substituted with one or more of halo, alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, CO_2R, C(O)R, CN, CF_3, NR_2, NO_2, and OR;

R^{18} is selected from the group consisting of H, alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, CF_3, aryl, and a heterocyclic ring;

each R is independently selected from H, alkyl, cycloalkyl, aralkyl, aryl and a heterocyclic ring; and

n is 0 to 6.

[0038] In a further preferred embodiment, the present invention provides inhibitors of the $P210^{BCR\text{-}ABL\text{-}T315I}$ theramutein having the formula III_a



(III_a)

X is N or C-R^1;

ring A is a 5-, 6-, or 7- membered heterocyclic ring or a 7- to 12-membered fused heterobicyclic ring;

the dotted lines represent optional double bonds,

each R^1 is independently selected from the group consisting of alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, CN, CF_3, NR_2, NO_2, OR, CO_2R, C(O)R, halo, aryl, and a heterocyclic ring, and additionally or alternatively, two R^1 groups on adjacent ring atoms form a fused 5- or 6-membered fused ring which contains from 0 to 3 heteroatoms;

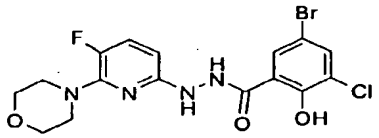
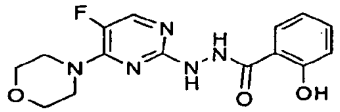
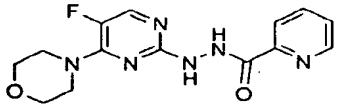
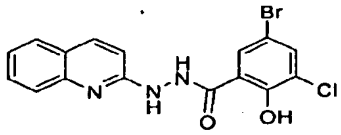
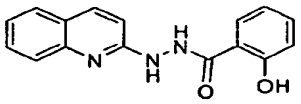
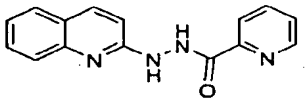
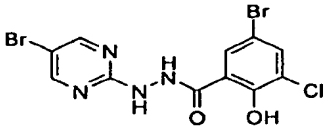
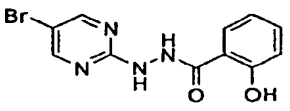
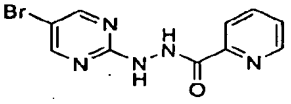
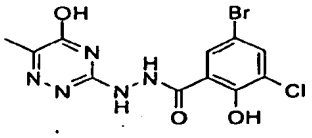
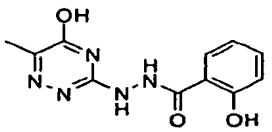
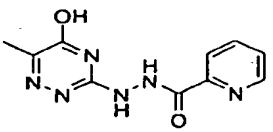
n is 0 to 6,

B is N, CH or C-R^{20};

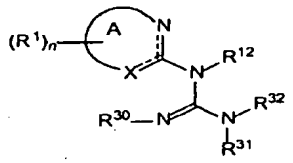
each R^{20} is independently selected from the group consisting of alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, CN, CF_3, NR_2, NO_2, OR, CO_2R, C(O)R, halo, aryl, and a heterocyclic ring, and additionally or alternatively, two R^{20} groups on adjacent ring atoms form a fused 5- or 6-membered fused ring which contains from 0 to 3 heteroatoms; and

m is 0 to 6.

[0039] Exemplary compounds of the formula III or III_a includes the following structures:

[0040] In a further embodiment, the present invention provides inhibitors of the $P210^{BCR\text{-}ABL\text{-}T315I}$ theramutein having the formula IV



(IV)

wherein:

ring A is a 5-, 6-, or 7- membered heterocyclic ring or a 7- to 12-membered fused heterobicyclic ring;

X is N or C-R^1;

the dotted lines represent optional double bonds;

each R^1 is independently selected from the group consisting of H, alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, CN, CF_3, NR_2, NO_2, OR, CO_2R, C(O)R, halo, aryl, and a heterocyclic ring, and additionally or alternatively, two R^1 groups on adjacent ring atoms form a 5- or 6-membered fused ring which contains from 0 to 3 heteroatoms;

n is 0 to 6;

R^{12} is selected from H and $C_{1\text{-}3}$ alkyl;

R^{30} is selected from H, alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, aryl, and a heterocyclic ring;

R^{31} is selected from H, alkyl, cycloalkyl, -(C=O)R, alkenyl, alkynyl, aralkyl, aryl, and a heterocyclic ring;

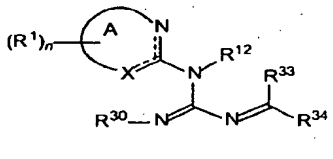
R^{32} is selected from -Y"-R^{13};

Y" is selected from a chemical bond, and an alkylene chain having from 1 to 4 carbon atoms, and optionally substituted with one or more of halo, alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, CO_2R, C(O)R, CN, CF_3, NR_2, NO_2, and OR;

R^{13} is selected from the group consisting of H, alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, CF_3, aryl, and a heterocyclic ring; and

each R is independently selected from H, alkyl, cycloalkyl, aralkyl, aryl and a heterocyclic ring.

[0041] In a further embodiment, the present invention provides inhibitors of the $P210^{BCR\text{-}ABL\text{-}T315I}$ theramutein having the formula V



(V)

wherein:

ring A is a 5-, 6-, or 7- membered heterocyclic ring or a 7- to 12-membered fused heterobicyclic ring;

X is N or C-R^1;

the dotted lines represent optional double bonds;

each R^1 is independently selected from the group consisting of H, alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, CN, CF_3, NR_2, NO_2, OR, CO_2R, C(O)R, halo, aryl, and a heterocyclic ring, and additionally or alternatively, two R^1 groups on adjacent ring atoms form a 5- or 6-membered fused ring which contains from 0 to 3 heteroatoms;

n is 0 to 6;

R^{12} is selected from H and $C_{1\text{-}3}$ alkyl;

R^{30} is selected from H, alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, aryl, and a heterocyclic ring;

R^{33} is selected from H, alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, aryl, and a heterocyclic ring;

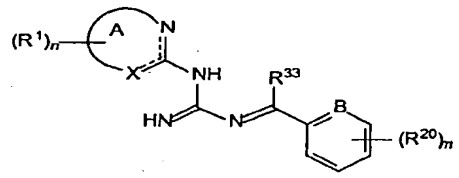
R^{34} is selected from -Y"-R^{13};

Y" is selected from a chemical bond, and an alkylene chain having from 1 to 4 carbon atoms, and optionally substituted with one or more of halo, alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, CO_2R, C(O)R, CN, CF_3, NR_2, NO_2, and OR;

R^{13} is selected from the group consisting of H, alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, CF_3, aryl, and a heterocyclic ring; and

each R is independently selected from H, alkyl, cycloalkyl, aralkyl, aryl and a heterocyclic ring.

[0042] In a further embodiment, the present invention provides inhibitors of the $P210^{BCR\text{-}ABL\text{-}T315I}$ theramutein having the formula V_a



(V_a)

wherein:

ring A is a 5-, 6-, or 7- membered heterocyclic ring or a 7- to 12-membered fused heterobicyclic ring;

X is N or C-R^1;

the dotted lines represent optional double bonds;

each R^1 is independently selected from the group consisting of H, alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, CN, CF_3, NR_2, NO_2, OR, CO_2R, C(O)R, halo, aryl, and a heterocyclic ring, and additionally or alternatively, two R^1 groups on adjacent ring atoms form a 5- or 6-membered fused ring which contains from 0 to 3 heteroatoms;

n is 0 to 6;

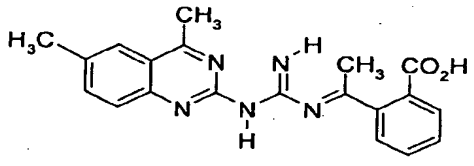
R^{33} is selected from H, alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, aryl, and a heterocyclic ring;

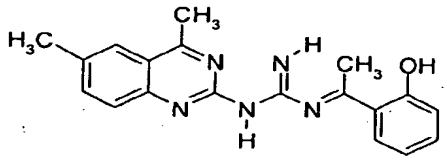
B is N or C-R^{20};

each R^{20} is independently selected from the group consisting of alkyl, cycloalkyl, alkenyl, alkynyl, aralkyl, CN, CF_3, NR_2, NO_2, OR, CO_2R, C(O)R, halo, aryl, and a heterocyclic ring, and additionally or alternatively, two R^{20} groups on adjacent ring atoms form a fused 5- or 6-membered fused ring which contains from 0 to 3 heteroatoms; and

m is 0 to 6.

[0043] Exemplary compounds of the formula V or V_a include the following structures:



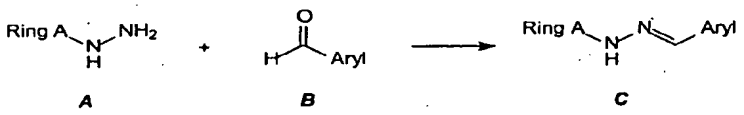


[0044] As used herein, the definition of each expression, e.g. alkyl, m, n, R, R' etc., when it occurs more than once in any structure, is intended to be independent of its definition elsewhere in the same structure.

[0045] It will be understood that chemical structures provided herein include the implicit proviso that substitution is in accordance with permitted valence of the substituted atom and the substituent(s), and that the substitution results in a stable compound, e.g., which does not spontaneously undergo transformation such as by rearrangement, cyclization, elimination, etc.

[0046] The compounds of the invention may generally be prepared from commercially available starting materials and known chemical techniques. Embodiments of the invention may be synthesized as follows. One of skill in the art of medicinal or synthetic chemistry would be readily familiar with the procedures and techniques necessary to accomplish the synthetic approaches given below.

[0047] Embodiments wherein $R^2 = NH$, $R^3 = N$, $R^4 = CH$, and $R^5 =$ -aryl may be prepared by reaction of an appropriate hydrazine compound, such as ***A***, and an appropriate aldehyde, such as ***B***, under conditions similar to those described on p. 562 of Gineinah, *et al.* (Arch. Pharm. Med. Chem. **2002**, 11, 556-562).

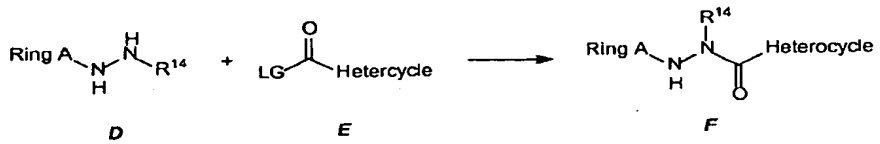


A ***B*** ***C***

For example, heating ***A*** with 1.1 equivalents of ***B*** for 1 to 24 hours in a protic solvent such as a C_1 to C_6 alcohol, followed by cooling and collection of the precipitate, would afford ***C***. Alternatively, product ***C*** may be isolated by evaporation of the solvent and purification by chromatography using silica gel, alumina, or C_4 to C_{18} reverse phase medium. Similar methodology would be applicable in the cases where "Aryl" is replaced by other groups defined under R^5.

[0048] Embodiments wherein $R^2 = NH$, $R^3 = NR^{14}$, $R^4 = C(O)$, and $R^5 =$ a heterocyclic ring may be prepared by reaction of an appropriate hydrazine compound, such as ***D***, and an activated carboxylic acid such as ***E***, wherein LG is a leaving group such as halo, 1-oxybenztriazole, pentafluorophenoxy, *p*-nitrophenoxy, or the like, or Compound ***E*** may also

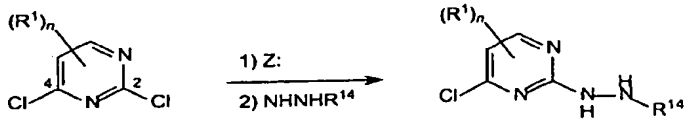
be a symmetrical carboxylic acid anhydride, whereby conditions similar to those described on p. 408 of Nair and Mehta (Indian J. Chem. **1967** 5, 403-408) may be used.



D *E* *F*

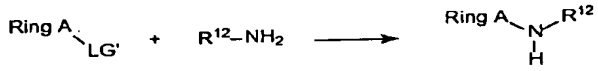
For example, treatment of ***D*** with an active ester such as Heterocycle-C(O)-OC_6F_5 in an inert solvent such as dichloromethane, 1,2-dichloroethane, or N,N-dimethylformamide, optionally in the presence of a base such as pyridine or another tertiary amine, and optionally in the presence of a catalyst such as 4-*N,N*-dimethylaminopyridine, at an appropriate temperature ranging from 0° C to the boiling point of the solvent, would afford ***F***, which may be isolated by evaporation of the solvent followed by chromatography using silica gel, alumina, or C_4 to C_{18} reverse phase medium. The above active ester example of ***E*** would be readily prepared from the corresponding carboxylic acid and pentafluorophenol using a carbodiimide such as dicyclohexylcarbodiimide as a condensing agent. Similar methodology would be applicable in the cases where "Heterocycle" is replaced by other groups defined under R^5.

[0049] Precursors such as ***A*** and ***D*** may be prepared by reaction of an appropriate nucleophile, for example, a hydrazine derivative, with a heteroaromatic compound bearing a halo substituent at a position adjacent to a nitrogen atom. For example, using methods analogous to those described by Wu, *et al.* (J. Heterocyclic Chem. **1990**, 27, 1559-1563), Breshears, *et al.* (J. Am. Chem. Soc. **1959**, 81, 3789-3792), or Gineinah, *et al.* (Arch. Pharm. Med. Chem. **2002**, 11, 556-562), examples of compounds ***A*** and ***D*** may be prepared starting from, for example, a 2,4-dihalopyrimidine derivative, many of which are commercially available or are otherwise readily prepared by one skilled in the art. Thus, treatment of an appropriate 2,4-dihalopyrimidine derivative ***G*** with an amine or other nucleophile (Z), optionally in the presence of an added base, selectively displaces the 4-halo substituent on the pyrimidine ring. Subsequent treatment of the product with a second nucleophilic reagent such as hydrazine or a hydrazine derivative, optionally in a solvent such as a C_1 to C_6 alcohol and optionally in the presence of an added base, displaces the 2-halo substituent on the pyrimidine ring, to afford compounds that are examples of structures ***A*** and ***D*** above.



G

[0050] Embodiments wherein R^2 is $-NR^{12}$ and R^3 is $-C(=R^{17})$ can be synthesized by methods such as the following, or straightforward modifications thereof. The synthesis may be conducted starting from an appropriate ring A derivative ***J*** that bears a leaving group (LG) adjacent to the requisite ring nitrogen. Structure ***G*** above and the product of reaction of structure ***G*** with nucleophile Z, as illustrated above, are examples of such appropriate Ring A derivatives ***J***. Suitable LG' groups are halo, alkylthio, alkylsulfonyl, alkylsulfonate or arylsulfonate. Treatment of ***J*** with an amine $R^{12}NH_2$ effects displacement of LG' to afford intermediates ***K***. An example of this chemical transformation wherein R^{12} is H and LG' is CH_3SO_2- is reported by Capps, *et al.* J. Agric. Food Chem. **1993**, 41, 2411-2415, and an example wherein R^{12} is H and LG' is Cl is reported in Marshall, *et al.* J. Chem. Soc. **1951**, 1004-1015.

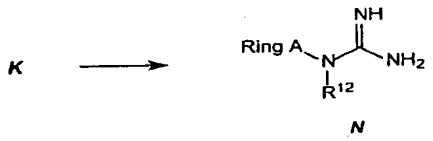


J *K*

[0051] Intermediates of structure ***K*** are transformed to compounds of the invention by simultaneous or sequential introduction of the elements, of R^3, R^4, and R^5. For example, treatment of intermediates of structure ***K*** with individual isocyanates R^6-N=C=O affords in a single step compounds of structure ***M***, which are compounds of the invention wherein R^2 = -NR^{12}-, R^3 = -C=O- , R^4 = -NH-, and R^5 = -chemical bond-R^6. Alternative methods to convert compounds of structure ***K*** to compounds of structure ***M*** are well known to those skilled in the art, wherein R^3 together with a leaving group (for example *p*-nitrophenoxy or chloro) is first introduced, followed by subsequent displacement of the leaving group by, for example, an amine R^6-NH_2, to introduce R^5 and R^6 .

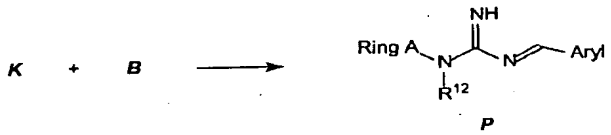
M

[0052] Alternatively, treatment of intermediates of structure ***K*** with a reagent such as cyanamide (NH_2-CN), typically under conditions of heating and optionally in the presence of acid in a solvent such as ethyl acetate or dioxane, affords intermediates ***N***. Alternatives to cyanamide are nitroguanidine or amidinosulfonic acid (NH_2-C(=NH)-SO_3H). An example of such a transformation using cyanamide is reported by Latham *et al.*, J. Org. Chem. **1950**, 15, 884. An example using nitroguanidine is reported by Davis, Proc. Natl. Acad. Sci. USA **1925**, 11, 72. Use of amidinosulfonic acid was reported by Shearer, *et al.* Bioorg. Med. Chem. Lett. **1997**, 7, 1763.



N

[0053] In analogy to the conversion of intermediates ***A*** or ***D*** to embodiments represented by ***C*** or ***F***, intermediates ***K*** are converted, respectively, to compounds represented by ***P*** or ***Q***, which are further embodiments of the invention.

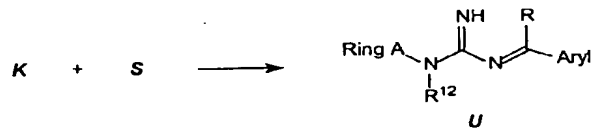


P

Q

[0054] Treatment of ***A*** or ***K*** with a ketone ***S***, wherein R is as defined above, in place of an aldehyde ***B*** in the schemes above, affords compounds of structure ***T*** or ***U***, respectively, which are further embodiments of the invention.

S ***T***



[0055] It will be apparent to a practitioner skilled in the art of organic molecule synthesis that the reaction processes illustrated above are representative of a broader set of methods that are logical extensions of the illustrated processes. Thus, additional embodiments of the invention that incorporate additional variants in R^2, R^3, R^4, and R^5 claimed by this invention are prepared by obvious modifications of the above processes.

[0056] As would be recognized by a person of ordinary skill, it may be advantageous to employ a temporary protecting group in achieving the final product. The phrase "protecting group" as used herein means temporary modifications of a potentially reactive functional group which protect it from undesired chemical transformations. Examples of such protecting groups include esters of carboxylic acids, silyl ethers of alcohols, and acetals and ketals of aldehydes and ketones, respectively. The field of protecting group chemistry has been reviewed (Greene, T. W.; Wuts, P. G. M. *Protective Groups in Organic Synthesis*, 2nd ed.; Wiley: New York, 1991).

[0057] A "mutein" is a protein having an amino acid sequence that is altered as a result of a mutation that has occurred in its corresponding gene (Weigel et al, 1989). Such mutations may result in changes in one or more of the characteristics of the encoded protein. For example, an enzyme variant that has modified catalytic activity resulting from a change in one or more amino acids is a mutein.

[0058] This invention is concerned with proteins harboring an alteration of at least one amino acid residue (the terms "amino acid sequence change" or "amino acid sequence alteration" include changes, deletions, or additions, of at least one amino acid residue, or any combination of deletions, additions, changes) such that the resulting mutein has become (as a result of the mutation) resistant to a known therapeutic agent relative to the sensitivity of the non-mutated version of said protein to the therapeutic agent. This specialized class of muteins is hereinafter referred to as a ***theramutein***, and the corresponding protein lacking the mutation is referred to herein as a ***prototheramutein***.

[0059] As used herein, "prototheramutein" refers to an endogenously occurring protein in a cell that is susceptible to mutation that confers relative insensitivity (i.e.

resistance) to a therapeutic compound which otherwise inhibits or activates the protein. Accordingly, "theramutein" refers to an endogenously occurring protein or portion of a protein in a cell that contains at least one amino acid sequence alteration relative to an endogenous form of the protein, wherein the amino acid sequence change is or was identified or becomes identifiable, and is shown to be clinically significant for the development and progression of a given disease, *following exposure of at least one human being to a substance that is known to inhibit or activate the prototheramutein*. Thus, ***by definition***, a theramutein is limited to a protein which harbors a mutation in its corresponding endogenous gene, wherein said mutation has been shown to be associated with the development of clinical resistance in a patient to a drug that is normally able to activate or inhibit the non-mutated protein. Accordingly, it is apparent to a skilled artisan that, as the genes which encode theramuteins are limited to endogenously occurring, the definition of a theramutein excludes proteins encoded by disease-causing infectious agents such as viruses and bacteria. As used herein, the term "endogenous gene" refers to a gene that has been present in the chromosomes of the organism, at least in its unmutated form, since inception. The term "cell" as used herein refers to a living eukaryotic cell whether in an organism or maintained under appropriate laboratory tissue or organ culture conditions outside of an organism.

[0060] In one aspect of the invention, a theramutein is a protein that is altered for the first time with respect to a commonly occurring "wild type" form of the protein (i.e. the prototheramutein). In another aspect of the invention, a theramutein is a variant of a protein (prototheramutein) that is, itself, already a mutein. In still another embodiment, a theramutein may be further mutated as compared to a previously existing theramutein. In such instances, the first theramutein (such as the T315I mutant of p210 BCR-ABL (see below), may be thought of as a "primary" theramutein, whereas subsequent mutations of the (already mutated) T315I variant may be termed a secondary theramutein, tertiary theramutein, etc. As exemplified below, a mutein of the invention is a variant of Bcr-Abl tyrosine kinase that escapes inhibition by an inhibitor of the "wild type" Bcr-Abl. Such a Bcr-Abl mutein is altered with respect to a more common or "wild type" form of Bcr-Abl (which is also a mutein as well) in such a way that a property of the protein is altered.

[0061] It will be understood that a mutein of primary interest is a theramutein that may have the same, increased, or decreased specific activity relative to its prototheramutein, and that it is not inhibited or is poorly inhibited by an agent that is capable of inhibiting the

prototheramutein. Likewise, another theramutein of primary interest is one that has the same, increased or decreased specific activity (relative to its prototheramutein) and that is not activated or is poorly activated by an agent that is capable of activating the prototheramutein. Other variations are obvious to the skilled artisan. It will be further appreciated that theramuteins can include naturally occurring or commonly observed variants of a protein, for example, variants that are expressed from different alleles of a particular gene. In some cases such variants may be unremarkable with respect to their normal cellular function, with functional differences becoming apparent only in the presence of agents that differentially inhibit or activate the cellular function of the variants. For example, naturally occurring variants of a particular enzyme may have activity profiles that are not substantially different, but a therapeutic agent that modulates one may be ineffective in modulating the other.

[0062] It will be appreciated that, whereas one aspect of the invention is an agent that is active against a theramutein that arises or becomes dominant (by any mechanism) during the course of a treatment for a given disease, another aspect is an agent that is active against a mutein that is common within a population of unafflicted individuals, but wherein said mutein is less susceptible to modulation by an approved drug, and where the variation in the activity profile of the mutein becomes important (and is therefore first identified as being a theramutein) in a disease state such as where it is overexpressed or participates in a signaling process which has otherwise become abnormally regulated. For example, a neoplastic disease may be caused by abnormal regulation of a cellular component other than the theramutein or its prototheramutein, and still be treatable with an inhibitor of the prototheramutein, whereas the same treatment would be less effective or ineffective where the theramutein was present. This can be an issue where it is observed that the response of a particular tumor type to an anticancer agent varies among individuals that express different variants of an enzyme against which the anticancer agent is directed (Lynch et al., 2004). Here, the variants would not have arisen or become predominant during the course of treatment of the disease, but are preexisting in the healthy population and are detected only by their altered responsiveness to a particular course of established therapeutic treatment.

[0063] As used herein, the terms "agonist" and "activator" of a protein are used interchangeably. An activator (agonist) is limited to a substance that binds to and activates the functioning of a given protein. Unless explicitly stated otherwise, an "activator", an "agonist", and an "activator of a protein" are identical in meaning. The activation by an

activator may be partial or complete. Likewise, as used herein, the terms "antagonist" and "inhibitor" of a protein are used interchangeably. An inhibitor (antagonist) is limited to a substance that binds to and inhibits the functioning of a given protein. Unless explicitly stated otherwise, an "inhibitor", an "antagonist" and an "inhibitor of a protein" are also synonymous. The inhibition by an inhibitor may be partial or complete. A modulator is an activator or an inhibitor. By way of example, an "activator of $PKC_{\beta 1}$" should be construed to mean a substance that binds to and activates $PKC_{\beta 1}$. Similarly, an "inhibitor of $p210^{Bcr\text{-}Abl}$," is a substance that binds to and inhibits the functioning of $p210^{Bcr\text{-}Abl}$. To state that a substance "inhibits a protein" means that the substance binds to the protein in order to exert it inhibitory effect. Similarly, to state that a substance "activates protein X" is to state that the substance binds to and activates protein X. The terms "binds," "binding," and "binds to" have their ordinary meanings in the field of biochemistry in terms of describing the interaction between two substances (*e.g.*, enzyme-substrate, protein-DNA, receptor-ligand, etc.). As used herein, the term "binds to" is synonymous with "interacts with" in the context of discussing the relationship between a substance and its corresponding target protein. As used herein, to state that a substance "acts on" a protein, "affects" a protein, "exerts its effect on" a protein, etc., and all such related terms uniformly mean (as the skilled investigator is well aware) that said substance activates or inhibits said protein.

[0064] The concept of inhibition or activation of a mutated form of an endogenous protein to an equivalent or greater extent than the corresponding non-mutated counterpart protein is defined for the first time and referred to herein as a positive "***specificity gap***." In general terms, *and using an inhibitor case as an example*, the ***specificity gap*** refers to the difference between the ability of a given substance, under comparable conditions to inhibit the theramutein in a cell-based assay system as compared to either:

a) the ability of the same substance under comparable conditions to inhibit the prototheramutein, or

b) the ability of a second substance (usually a known inhibitor of the prototheramutein) to inhibit the theramutein under comparable conditions, or

c) the ability of the second substance to inhibit the prototheramutein under comparable conditions.

[0065] When the comparison is made between the effects of two distinct substances (tested individually) on the theramutein alone, the result is termed a ***homologous specificity gap*** determination.

[0066] Alternatively, when a comparison is made between the effects of two distinct substances, one of which is tested on the theramutein and the other on the prototheramutein, respectively, the result is termed a ***heterologous specificity gap*** determination. Thus, (a) and (c) as given above are examples of heterologous SG determinations, whereas (b) is an example of a homologous SG determination.

[0067] Reference to Figure 2 is informative in understanding and elucidating these concepts.

[0068] Analogous issues apply when the case concerns an activator. It will be immediately obvious to the skilled artisan that the term "comparable conditions" includes testing two different compounds, for example, at the same concentration (such as comparing two closely related compounds to determine relative potency), or by comparing the effects of two different compounds tested at their respective IC_{50} values on the corresponding prototheramutein and theramutein.. The skilled investigator will easily recognize other useful variations and comparable conditions.

[0069] Thus, in one embodiment of the application of this approach, substances that are more effective against a theramutein have a "positive specificity gap." A "zero, null or no" specificity gap indicates that there is no significant measurable difference between the effect of a substance on the theramutein as compared to its effect on the prototheramutein, (however such compounds may be quite useful in their ability to inhibit or activate both a theramutein and its corresponding prototheramutein), and a "negative specificity gap" indicates a substance that at a given concentration is less effective against the given theramutein than against the unmutated endogenous or other comparative form of the theramutein (or the corresponding prototheramutein). The latter category is generally of lesser interest than the former categories of compounds, except in the case where the compound is so potent that its relatively lesser effect on the theramutein is of no real concern from the perspective of therapeutic efficacy. The skilled investigator can easily recognize a variety of approaches to quantifying the specificity gap assessment in a manner tailored to his or her needs.

[0070] The invention also provides a means for identifying compounds that exhibit a desired specificity gap. Such compounds can be identified and their ability to inhibit or activate the theramutein determined using an *in vitro* cell-based assay system where the effect of a substance on the cellular functioning of the mutated endogenous form of the protein is compared to the effect of the same drug on the cellular functioning of a non-mutated endogenous form of the protein.

[0071] Thus, the system enables the discovery of compounds capable of binding to a theramutein and exerting a greater modulatory effect on the cellular functioning of said theramutein than on its corresponding prototheramutein. Further, the system enables the discovery of compounds capable of binding to a theramutein and exerting at least as great or greater modulatory effect on the cellular functioning of a theramutein than previously known compounds are able to exert on the corresponding prototheramutein. In a particular embodiment of the invention, a compound may be screened for and identified that 1) is at least as effective against the theramutein as the original drug is against the prototheramutein, and 2) is similarly effective against the prototheramutein as against the theramutein (*i.e.*, displays a small or essentially zero specificity gap).

[0072] In an embodiment of the invention, cells that overexpress a theramutein of interest are used to identify chemical agents that are inhibitors or activators of (*i.e.*, that bind to and inhibit or that bind to and activate) at least the selected theramutein. The chemical agents may also be inhibitors or activators of the prototheramutein or even other theramuteins of the same prototheramutein. As used herein, the terms "chemical agent" and "compound" are used interchangeably, and both terms refer exclusively to substances that have a molecular weight up to, but not including 2000 atomic mass units (Daltons). Such substances are sometimes referred to as "small molecules." Unless otherwise stated herein, the term substance as used herein refers exclusively to chemical agents/compounds, and does not refer to ***biological agents***. As used herein, "***biological agents,***" are molecules which include proteins, polypeptides, and nucleic acids, and have molecular weights equal to or greater than 2000 atomic mass units (Daltons).

[0073] According to the invention, a theramutein is selected and used in a cell-based assay system designed to identify agents that are inhibitors or activators of the theramutein. Where two or more distinct theramuteins originating from the same prototheramutein are known, it is strongly advisable to select the most resistant theramutein available for use in the

assay system. In general, the degree of resistance of a theramutein to a given chemical agent is determined relative to its non-mutated counterpart (prototheramutein) using the drug that was first administered and known to inhibit or activate the prototheramutein and against which the theramutein "arose." The methods of determining the degree of such resistance, for example by analysis of IC_{50} or AC_{50} values, are well known in the art and will not be reiterated herein. However, no causal relationship is necessary or should be inferred between the treatment of the patient with a given therapeutic agent per se and the subsequent appearance of a theramutein. Rather, what is required in order to practice the invention is that a true theramutein be properly selected according to the teachings herein.

[0074] Thus, for example, randomly generated site directed mutants of known proteins that are created in the laboratory but that have ***not*** been shown to be clinically relevant are not appropriate muteins for use within the scope of this invention. Such muteins would not, of course, be properly classified as theramuteins.

[0075] For example, in an effort to obtain potential inhibitors of mutants of $p210^{Bcr\text{-}Abl}$, Huron et al. (2003) used a recombinant c-abl preparation and screened a series of compounds known to inhibit c-src tyrosine kinase activity. The authors performed c-abl kinase assays on their compounds and identified the most potent compound as an 8 nM inhibitor against c-abl. When this compound (PD166326) was tested against various $p210^{Bcr\text{-}Abl}$ theramuteins, however, it showed activity against some of the mutants such as $p210^{Bcr\text{-}Abl\text{-}E255K}$, but the $p210^{Bcr\text{-}Abl\text{-}T315I}$ theramutein was found to remain 10 fold more resistant (Huron et al. 2003, Table 3). Furthermore, in each case the compound was still markedly ***less effective*** on the $p210^{Bcr\text{-}Abl}$ theramuteins than it was against the wild-type $p210^{Bcr\text{-}Abl}$. When the compound was tested against $p210^{Bcr\text{-}Abl\text{-}T315I}$ mutant activity, it was unable to inhibit the activity to any appreciable extent (p. 1270, left hand column, second paragraph; see also Fig. 4.). Thus, the disclosed compound was able to inhibit a theramutein that is partially resistant to STI-571, but had no activity against the T315I mutant of Bcr-Abl., which was already known at that time to be the theramutein that exhibited the most resistance to STI-571. Hence purely and simply, the Huron methodology failed to identify an effective inhibitor of the $p210^{Bcr\text{-}AblT315I}$ theramutein.

[0076] Indeed, prior to the disclosure of this invention, including both the detailed methodology described for the first time herein as well as the compositions provided herein, *no one anywhere in the world* has been successful in identifying a chemical agent, let alone

a methodology, capable of identifying a chemical agent that effectively inhibits the $p210^{Bcr\text{-}AblT315I}$ theramutein to an equal or greater extent than STI-571 is able to do with respect to the wild type $p210^{Bcr\text{-}Abl}$ protein. (See Shah et al., Science, August, 2004; O'Hare et al., Blood, 2004; Tipping et al., Leukemia, 2004; Weisberg et al., Leukemia, 2004).

[0077] It cannot be overemphasized that such compounds would be immensely useful, because at the present time there is no alternative for patients who progress to $p210^{Bcr\text{-}Abl\text{-}T315I}$ theramutein-mediated imatinib mesylate-resistant status. *Once patients develop such resistance, there is no other effective alternative treatment available, and death is certain. The method described herein provides the first reported approach to identify, pharmacologically characterize and chemically synthesize effective inhibitors* of the $p210^{Bcr\text{-}Abl\text{-}T315I}$ *theramutein. Moreover, the skilled investigator will immediately recognize the applicability and generalizability of this approach to any highly drug-resistant theramutein.*

[0078] In the present invention, a test cell is used that displays a phenotypic characteristic (as defined below) which is linked to the presence and functional activity of the particular theramutein-of-interest (TOI) in the cell under appropriate conditions. This may be the same as a phenotypic characteristic displayed by a cell that expresses the prototheramutein. A phenotypic characteristic (i.e. a non-genotypic characteristic of the cell) is a property which is observed (measured), selected and/or defined for subsequent use in an assay method as described herein. Expression of the phenotypic characteristic is responsive to the total activity of the theramutein in the cell, and is a result of the absolute amount of the theramutein and its specific activity. Often, the phenotypic characteristic is observable as a result of elevated levels of theramutein activity and is not apparent in cells that express low amounts of the theramutein or low amounts of its corresponding prototheramutein. Further, it can often be demonstrated that the phenotypic characteristic is modulated by modulating the specific activity of the theramutein with an inhibitor or activator of the theramutein, although this is not always the case since an inhibitor or activator of the TOI may not always be available at the time the skilled investigator undertakes such a project. Thus, for the purpose of defining the phenotypic characteristic to be subsequently used with a given test cell for assay purposes, the skilled investigator may also use a substance capable of increasing or decreasing the expression of the theragene, which will in turn lead to increases or decreases of the level of the corresponding theramutein. This allows the skilled investigator to simulate the effects of certain types of activators or inhibitors of the theramutein (such as a suicide

inhibitor of the theramutein, which is a class of chemical agent which binds irreversibly and covalently modifies the TOI, rendering it permanently inactive), without actually having access to such a compound, for the purposes of refining the appropriate phenotypic characteristic for subsequently establishing a useful cellular assay system. Examples known to one of ordinary skill that would be helpful for such purposes include the use of anti-sense DNA oligonucleotides, small interfering RNAs, other RNA interference-based methodologies, and vector constructs containing inducible promoter systems. In this manner, the selected phenotypic characteristic is linked to the activity of the theramutein in the test cell. Notably for theramuteins, the selected phenotypic characteristic is usually also displayed by a cell that over expresses the prototheramutein and in which the phenotypic characteristic is modulated by known inhibitors or activators of the prototheramutein.

[0079] A phenotypic characteristic is simply a characteristic of a cell or organism other than a genotypic characteristic of the cell. Except for the specific requirements of a properly defined phenotypic characteristic as disclosed herein for the purposes of creating useful cellular assay systems according to the teachings of certain of the embodiments of the invention, no other limitation of the term phenotypic characteristic of any kind or nature is intended or appropriate in order to properly and effectively practice the invention. Indeed, the skilled artisan must be able to select any characteristic of the cell that maximizes the utility of establishing the proper cell-based assay for his or her needs. The phenotype characteristic can be quantitative or qualitative and be observable or measurable directly (*e.g.*, observable with the naked eye or with a microscope), but most commonly the characteristic is measured indirectly using standard automated laboratory equipment and assay procedures which are known to those of skill in the art. The term "observable" means that a characteristic may be measured or is otherwise detectable under appropriate conditions by any means whatsoever, including the use of any type of laboratory instrumentation available. The term "detectable" is not the same as "detected". A characteristic may be detectable to a skilled artisan without being detected at any given time, depending upon how the investigator chooses to design the assay system. For example, in searching for activators of a prototheramutein (or theramutein), it may be desirable to have the relevant phenotypic characteristic detected only after the addition of a known activator or test substance capable of activating the POI. This provides the ability to maximize the intensity of the signal that is generated by the test cell in the assay.

[0080] Phenotypic characteristics include but are not limited to growth characteristics, transformation state, differentiation state, substrate phosphorylation state, catalytic activity, ion flux across the cell membrane (calcium, sodium, chloride, potassium, hydrogen ions, etc.), pH changes, fluctuations of second messenger molecules or other intracellular chemical species such as cAMP, phosphoinositides, cyclic nucleotides, modulations of gene expression, and the like. The characteristic of the cell may be observable or measurable continuously (*e.g.*, growth rate of a cell), or after a period of time (*e.g.*, terminal density of a cell culture), or transiently (*e.g.*, modulation of a mutein causes a transient change in phosphorylation of a substrate of the mutein, or a transient flux in ion flow across the membrane, or elevations or reductions in intracellular cAMP levels). In certain embodiments, a selected phenotypic characteristic may be detectable only in the presence of a modulator of the prototheramutein or the theramutein. No limitations are intended with respect to a characteristic that may be selected for measurement. As used herein, the terms "characteristic of a cell" and "phenotypic characteristic", and simply "characteristic", when used to refer to the particular measurable property of the intact cell or a subcellular fraction of the cell, are identical. For example, a phenotypic characteristic can be focus formation that becomes observable when a cell that over expresses a selected protein is cultured in the presence of an activator of the protein, or it may be a transient increase or decrease in the level of an intracellular metabolite or ion, such as cAMP, calcium, sodium, chloride, potassium, lithium, phosphoinositol, cGMP, bicarbonate, etc. It is obvious to one of ordinary skill in the art that after a cell is exposed to a test substance, the characteristic so measured (assayed) may be determined on a sub-cellular fraction of the cell. However, the initial treatment of the cell with a substance, which thereby causes the substance to come into contact with the cell, must be performed on the intact cell, not a sub-cellular fraction.

[0081] The characteristic selected for measurement within the cell must not be an intrinsic physical or chemical property of the theramutein or prototheramutein itself (such as the mere amount (mass) of the protein inside the cell), but rather must be a characteristic that results from the activity of the theramutein inside the cell, thus affecting a characteristic of the cell which is distinct from the theramutein itself, as discussed in detail above. For example, where the theramutein is a protein kinase that is capable of undergoing *autophosphorylation*, a process whereby the enzyme is capable of catalyzing the phosphorylation of itself by transferring a terminal phosphate group from ATP onto itself, it

would NOT be appropriate to select the phosphorylation state of the TOI as an appropriate phenotypic characteristic of the cell for measurement. This is because such a characteristic does not reflect the activity of the TOI on other cellular components. As the skilled investigator knows, autophosphorylation is not necessarily reflective of the activity of a protein kinase in a cell, since mutants of protein kinases are known that retain enzymatic activity sufficient to undergo autophosphorylation, yet have lost the capability to engage in signal transduction events within the cell. The classic paper by White et al. (1988) is both educational and noteworthy in this respect.

[0082] The term "responsive phenotypic characteristic" means a characteristic of the cell which is responsive to inhibitors or activators of a given protein (prototheramutein or theramutein). The term "known therapeutic agent" is defined as any agent that has been administered to a human being for the treatment of a disease in a country of the world.

[0083] A useful phenotypic characteristic, as exemplified herein in association with $p210^{Bcr\text{-}Abl}$ and theramuteins thereof, is disregulation of cell growth and proliferation. It is noted that the same or similar assay may be appropriate for use with many different proteins of interest. For example, disregulations of growth, proliferation, and/or differentiation are common phenotypic characteristics that may result from overexpression of a variety of different cellular proteins. By overexpressing a selected protein in order to cause the appearance of such a phenotypic characteristic, the characteristic becomes linked to the presence, amount, and specific activity of that selected protein under suitable conditions.

[0084] Though not always necessary, it will often be advantageous to employ cells that express high levels of the theramutein, and to select a phenotypic characteristic that results from overexpression of the theramutein. This is because phenotypic characteristics linked to the functioning of the theramutein generally become more distinguishable (easier to measure) as a theramutein is overexpressed to a greater extent. Further, changes (or responses) of the phenotypic characteristic that are observed in response to modulators of the theramutein are often amplified as the functional level of the theramutein is increased. Expressed another way, the selected (responsive) phenotypic characteristic observed in cells that overexpress the theramutein is particularly sensitive to modulators of the theramutein. The theramutein should be stably expressed. Stable expression results in a consistent level of the protein in the cell that generally does not change substantially during the course of an assay. For example, stimulation or activation of a component of a signaling pathway may be

followed by a refractory period during which signaling is inhibited due to down-regulation of the component. For theramuteins of the invention, such down-regulation is usually sufficiently overcome by artificially overexpressing the theramutein. Expressed another way, the expression is sufficiently maintained that changes in a phenotypic characteristic that are observed during the course of an assay are due primarily to inhibition or activation of the theramutein, even if down-modulation of the theramutein subsequently occurs For these reasons, although stable expression of the theramutein is preferred, transfection followed by transient expression of the theramutein may be employed provided that the phenoresponse is measurable and the duration of the assay system is short relative to the progressive decline in the levels of the transiently expressed theramutein that is to be expected in such systems over time.

[0085] A preferred drug screening method of the present invention involves the following:

[0086] 1) Identification of a theramutein for which a novel inhibitor or activator is desired. Identification of an appropriate theramutein may be performed using standard techniques (See Gorre et al., Science, 2001; see also PCT/US02/18729). Briefly, patients that have been given a course of therapeutically effective treatment using an activator or inhibitor of a known or suspected prototheramutein and have subsequently shown clinical signs and symptoms consistent with disease relapse are identified, and cells or tissue samples derived from such patients are obtained. Using standard laboratory techniques such as RT-PCR, the sequence of the prototheramutein is determined and compared to the previously determined nucleic acid sequence of the known prototheramutein gene or cDNA sequence. Mutations, if present, are identified and are correlated with functional resistance of the prototheramutein's function either in cell-based or, more commonly, cell-free assay systems, again using standard methodology. Once resistance-inducing mutations are confirmed, then said one or more confirmed mutants comprise a defined theramutein which may be used in the subsequent methods as described herein.

[0087] 2) Provision of a test cell that expresses a theramutein of interest and displays an observable (measurable) phenotypic characteristic which has been previously shown to be responsive to inhibitors or activators of the theramutein or, more commonly, the corresponding prototheramutein. Said specific phenotypic characteristic that has been previously shown to be responsive to inhibitors or activators of the theramutein-of-interest

(TOI), and/or the prototheramutein-of-interest (pTOI) is defined herein for the first time as a "**phenoresponse.**" One embodiment of this invention is the definitive use of the **phenoresponse** for the purpose of identifying compounds that are likely to be inhibitors or activators of the TOI. This may be accomplished through the use of a high-throughput screen using a cell line overproducing a given TOI and for which an appropriate phenoresponse has been identified and characterized. In one embodiment, a cell is selected that naturally expresses the theramutein such that a responsive phenotypic characteristic is present under suitable culture conditions which are obvious to one of ordinary skill in the art. In other embodiments, the theramutein is overexpressed, in some instances in a host cell that does not otherwise express the theramutein at all. This usually involves construction of an expression vector from which the theramutein can be introduced into a suitable host cell and overexpressed using standard vector systems and methodology. In one embodiment, overexpression results in a level of the theramutein that is at least about 3 times the amount of the protein usually present in a cell. Alternatively, the amount is at least about 10 times the amount usually present in a cell. In another embodiment, the amount is at least about 20 times or more preferably at least about 50 times the amount usually present in a cell.

[0088] 3) Provision of a control cell that expresses the corresponding prototheramutein of the theramutein of interest. As some of the muteins that are described herein are also enzymes, , they usually retain catalytic activity, and therefore the control cell usually displays substantially the same phenotypic characteristic as the test cell. The phenotypic characteristic need not be quantitatively alike in both cells, however. For example, a mutation that leads to reactivation of the prototheramutein may also increase, decrease, or otherwise affect its specific activity with respect to one or more of its substrates in the cell. As a result, it may exhibit the selected phenotypic characteristic to a greater or lesser extent. Accordingly, it may be desirable in some cases to adjust expression of either or both of the prototheramutein and the theramutein such that test and control cells exhibit the phenotypic characteristic to approximately the same degree. This may be done, for example, by expressing the proteins from promoters whose activity can be adjusted by adjusting the amount of inducer present, all using standard methodology (see, for example, Maniatis et al.)

[0089] The skilled investigator may also wish to use unmodified host cells or host cells harboring the expression vector only as control cells for certain experimental

procedures. (The host cells are the cells into which an expression vector encoding the theramutein was introduced in order to generate the test cells.

[0090] 4) The test and control cells are then maintained or propagated (although not necessarily at the same time) in growth media (or even in intact animals) under suitable conditions such that the selected phenotypic characteristic may be expressed and assayed. Control cells that are expressing the prototheramutein may be treated with a known modulator of the prototheramutein, or with a test substance, and test cells are treated with test compounds to determine whether they are active against the theramutein, as measured by the ability of said substances to modulate the selected phenotypic characteristic in the expected manner. Alternatively, control cells not expressing the prototheramutein may also be substituted, depending upon the particular phenoresponse that the skilled investigator has chosen for study. Substances may then be assayed on the test cells and, optionally, on the control cells at the same time, or at another time, and the results compared.

[0091] In one embodiment of the invention, substances that are active with regard to the test cells can be rapidly identified by their ability to modulate the phenoresponse of the test cells in the same manner as, for example, the known modulator of the prototheramutein alters the phenoresponse of prototheramutein-expressing control cells. In another embodiment, active substances may be identified by their ability to modulate the activity of the theramutein in the test cells while having little or no effect on the unmodified (prototheramutein and theramutein non-expressing) control cells. The skilled investigator will readily appreciate the many variations of this approach that may be utilized to identify, for example, modulators that are more effective against the theramutein, or that are equally effective against both the prototheramutein and one or more corresponding specific theramuteins.

[0092] Other phenoresponses can be observed and/or measured and include, for example, detection of substrates of the prototheramutein, and detection of gene expression changes that are regulated by the activity of the theramutein. In the simplest terms, any characteristic of the cell that the skilled investigator has previously correlated with the functional activity of the theramutein may be suitable for use with such methods. However, in selecting a given characteristic, the skilled investigator must first verify that said characteristic fulfills the criteria of being a phenoresponse according the teachings herein.

[0093] Characteristics suitable for detection may be measured by a variety of methods very well known to those of skill in the art. Such methods include, but are not limited to, detection of fluorescence of suitably labeled proteins (FACS), immunohistochemistry (IHC) for detection of protein expression, competitive radioligand binding assays, solid matrix blotting techniques, such as Northern, Southern and Western blots of cell extracts, reverse transcriptase polymerase chain reaction (RT-PCR), enzyme linked immunosorbent assays (ELISA), phosphorylation assays, gel retardation assays, membrane potential perturbations, and the like. The relevant phenotypic characteristic may be detected either on the intact cell after treatment with a test substance or, alternatively, on a subcellular fraction of the cell after treatment of the intact cell with a test substance.

[0094] Once compounds are identified that have the desired effect on the theramutein expressing test cells, it is desirable (but not necessary) to independently verify that the compounds identified are exerting their effects on the theramutein through a direct binding mechanism, i.e. that the compounds fulfill the criteria of being inhibitors or activators (as desired) of the theramutein according to the teachings of the invention (the reader is referred to the definitions of the terms "activator" and "inhibitor" as given above). This may be accomplished with numerous standard binding assays that are known to one of ordinary skill in the art, involving either purified protein samples or intact cellular binding assays using cells transfected with the appropriate prototheramutein or theramutein together with appropriate controls as dictated by sound scientific methods. Since such methods are well established in the art they will not be reiterated here. Numerous reference texts comprehensively discuss such techniques (see, for example, Foreman and Johansen, 2002; Enna S.J. et al. (1991) Current Protocols in Pharmacology, Wiley & Sons, Incorporated; Bonifacino, J.S. et al. (1999) Current Protocols in Cell Biology, Wiley & Sons, Incorporated). See also Housey, G.M. 1988, Chapter 4, and references therein; see also Horowitz et al., 1981.

[0095] In a particular embodiment of the invention, the method is used to identify substances that are inhibitors of the $p210^{Bcr\text{-}Abl\text{-}T315I}$ theramutein. The prototheramutein and theramutein are each expressed in Ba/F3 (murine) cells using standard methodology and the responsive phenotypic characteristics that are observed are growth characteristics (terminal cell density for a carefully defined cell culture, and growth in the absence of Interleukin-3

(IL-3). Unmodified host cells, or host cells containing the expression vector only, may optionally also be used.

[0096] Another useful assay is the determination of the state of phosphorylation of a direct substrate of $p210^{Bcr\text{-}Abl\text{-}T315I}$. One such substrate is Crkl (Gorre et al., *Science* 293:876-80 (2001)), an adapter protein which mediates the connection between Bcr-Abl and Ras. The phosphorylation state of CRKL is representative of the signaling activity of $p210^{Bcr\text{-}Abl}$ in a cell. Another downstream substrate is p62DOK. Any such substrate would suffice for these purposes, provided of course that phosphorylation of said substrate has been shown to occur inside the cell, and is not simply an autophosphorylation event of the TOI or PTOI as discussed above.

[0097] As exemplified herein, inhibitors of the T315I theramutein have been identified. Furthermore, these inhibitors are also active to differing extents against the wild type prototheramutein $p210^{Bcr\text{-}Abl\text{-}wt}$.

[0098] According to the present invention, a therapeutically effective amount of one or more compounds that modulate the functional activity of a $p210^{Bcr\text{-}Abl}$ theramutein is administered to a mammal in need thereof. The term "administering" as used herein means delivering the compounds of the present invention to a mammal by any method that may achieve the result sought. They may be administered, for example, orally, parenterally (intravenously or intramuscularly), topically, transdermally or by inhalation. The term "mammal" as used herein is intended to include, but is not limited to, humans, laboratory animals, domestic pets and farm animals. "Therapeutically effective amount" means an amount of a compound that, when administered to a mammal, is effective in producing the desired therapeutic effect, such as inhibiting kinase activity, inhibiting cancer cell growth and division, etc.

[0099] The invention provides a method of treating disease in a mammal by administering to the mammal an effective amount of a modulator of a theramutein. Suitable diseases to be treated according to the present invention include, but are not limited to, relapsing neoplastic or other proliferative disorders that have become resistant to previously administered drugs. The method is also useful for overcoming variation among individuals with respect to susceptibility to drug treatment that results from allelic differences among therapy targets. For example, the role of $p210^{Bcr\text{-}Abl}$ tyrosine kinase signaling in CML has been extensively demonstrated, as has the role of theramuteins of $p210^{Bcr\text{-}Abl}$ in drug resistant

recurrence of CML. Further, different muteins of $p210^{Bcr-Abl}$ exhibit varying sensitivity to inhibitors of $p210^{Bcr-Abl}$. Although some theramuteins arise during drug therapy, others may preexist in the population. These latter examples will not be recognized as theramuteins until such time as the disease state ensues and is followed by treatment with a known class of therapeutic agents Only after said treatment will such preexisting theramuteins reveal themselves as being clinically significant in terms of relative non-responsiveness leading to the progression of the disease in the patient harboring the theramutein.

[0100] In an embodiment of the invention, theramutein modulators are administered in combination with one or more other anti-neoplastic agents. Any suitable anti-neoplastic agent can be used, such as a chemotherapeutic agent, radiation or combinations thereof. The anti-neoplastic agent can be an alkylating agent or an anti-metabolite. Examples of alkylating agents include, but are not limited to, cisplatin, cyclophosphamide, melphalan, and dacarbazine. Examples of anti-metabolites include, but not limited to, doxorubicin, daunorubicin, and paclitaxel, gemcitabine, and topoisomerase inhibitors irinotecan (CPT-11), aminocamptothecin, camptothecin, DX-8951f, topotecan (topoisomerase I inhibitor) and etoposide (VP-16; topoisomerase II inhibitor) and teniposide (VM-26) (topoisomerase II inhibitor). When the anti-neoplastic agent is radiation, the source of the radiation can be either external (external beam radiation therapy – EBRT) or internal (brachytherapy – BT) to the patient being treated. The dose of anti-neoplastic agent administered depends on numerous factors, including, for example, the type of agent, the type and severity of the tumor being treated and the route of administration of the agent. It should be emphasized, however, that the present invention is not limited to any particular dose, route of administration, or combination of chemotherapeutic agents or other therapeutic regimens that are combined with the administration of theramutein modulators.

[0101] Anti-neoplastic agents which are presently known in the art or being evaluated can be grouped into a variety of classes including, for example, mitotic inhibitors, alkylating agents, anti-metabolites, intercalating antibiotics, growth factor inhibitors, cell cycle inhibitors, enzymes, topoisomerase inhibitors, anti survival agents, biological response modifiers, anti-hormones, and anti-angiogenesis agents, all of which can be administered with inhibitors or activators of theramuteins.

[0102] Modulators of theramuteins can be administered with antibodies that neutralize other receptors involved in tumor growth. In an embodiment of the invention, a

theramutein modulator is used in combination with a receptor antagonist that binds specifically to the Epidermal Growth Factor Receptor (EGFR). Particularly preferred are antigen-binding proteins that bind to the extracellular domain of EGFR and block binding of one or more of its ligands and/or neutralize ligand-induced activation of EGFR. An EGFR antagonist can be an antibody that binds to EGFR or a ligand of EGFR and inhibits binding of EGFR to its ligand. Ligands for EGFR include, for example, EGF, TGF-α, amphiregulin, heparin-binding EGF (HB-EGF) and betacellulin. EGF and TGF-α are thought to be the main endogenous ligands that result in EGFR-mediated stimulation, although TGF-α has been shown to be more potent in promoting angiogenesis. It should be appreciated that the EGFR antagonist can bind externally to the extracellular portion of EGFR, which can or can not inhibit binding of the ligand, or internally to the tyrosine kinase domain. Examples of EGFR antagonists that bind EGFR include, without limitation, biological agents such as antibodies (and functional equivalents thereof) specific for EGFR, and chemical agents (small molecules), such as synthetic kinase inhibitors that act directly on the cytoplasmic domain of EGFR.

[0103] Other examples of growth factor receptors involved in tumorigenesis are the receptors for vascular endothelial growth factor (VDGFR-1 and VEGFR-2), platelet-derived growth factor (PDGFR), nerve growth factor (NGFR), fibroblast growth factor (FGFR), and others.

[0104] In a combination therapy, the theramutein inhibitor is administered before, during, or after commencing therapy with another agent, as well as any combination thereof, *i.e.*, before and during, before and after, during and after, or before, during and after commencing the anti-neoplastic agent therapy. For example, the theramutein inhibitor can be administered between 1 and 30 days, preferably 3 and 20 days, more preferably between 5 and 12 days before commencing radiation therapy. In a preferred embodiment of the invention, chemotherapy is administered prior to, concurrently with or, more preferably, subsequent to antibody therapy.

[0105] In the present invention, any suitable method or route can be used to administer theramutein inhibitors of the invention, and optionally, to co-administer anti-neoplastic agents and/or antagonists of other receptors. The anti-neoplastic agent regimens utilized according to the invention, include any regimen believed to be optimally suitable for the treatment of the patient's neoplastic condition. Different malignancies can require use of

specific anti-tumor antibodies and specific anti-neoplastic agents, which will be determined on a patient to patient basis. Routes of administration include, for example, oral, intravenous, intraperitoneal, subcutaneous, or intramuscular administration. The dose of antagonist administered depends on numerous factors, including, for example, the type of antagonists, the type and severity tumor being treated and the route of administration of the antagonists. It should be emphasized, however, that the present invention is not limited to any particular method or route of administration.

[0106] Suitable carriers include, for example, one or more of water, saline, phosphate buffered saline, dextrose, glycerol, ethanol and the like, as well as combinations thereof. Carriers can further comprise minor amounts of auxiliary substances, such as wetting or emulsifying agents, preservatives or buffers, which enhance the shelf life or effectiveness of the theramutein modulator as the active ingredient. The compositions can, as is well known in the art, be formulated so as to provide quick, sustained or delayed release of the active ingredient after administration to the mammal.

[0107] The compositions of this invention can be in a variety of forms. These include, for example, solid, semi-solid and liquid dosage forms, such as tablets, pills, powders, liquid solutions, dispersions or suspensions, liposomes, suppositories, injectable and infusible solutions. The preferred form depends on the intended mode of administration and therapeutic application.

[0108] Such compositions of the present invention are prepared in a manner well known in the pharmaceutical art. In making the composition the active ingredient will usually be mixed with a carrier, or diluted by a carrier and/or enclosed within a carrier which can, for example, be in the form of a capsule, sachet, paper or other container. When the carrier serves as a diluent, it can be a solid, semi-solid, or liquid material, which acts as a vehicle, excipient or medium for the active ingredient. Thus, the composition can be in the form of tablets, lozenges, sachets, cachets, elixirs, suspensions, aerosols (as a solid or in a liquid medium), ointments containing, for example, up to 10% by weight of the active compound, soft and hard gelatin capsules, suppositories, injection solutions, suspensions, sterile packaged powders and as a topical patch.

[0109] It should be appreciated that the methods and compositions of the present invention can be administered to any suitable mammal, such as a rabbit, rat, or mouse. More preferably, the mammal is a human.

[0110] Throughout this application, various publications, reference texts, textbooks, technical manuals, patents, and patent applications have been referred to. The teachings and disclosures of these publications, patents, patent applications and other documents in their entireties are hereby incorporated by reference into this application to more fully describe the state of the art to which the present invention pertains.

[0111] It is to be understood and expected that variations in the principles of invention herein disclosed may be made by one skilled in the art and it is intended that such modifications are to be included within the scope of the present invention.

[0112] The following examples further illustrate the invention, but should not be construed to limit the scope of the invention in any way. Detailed descriptions of conventional methods, such as those employed in the construction of vectors and plasmids, the insertion of genes encoding polypeptides into such vectors and plasmids, the introduction of plasmids into host cells, and the expression and determination thereof of genes and gene products can be obtained from numerous publications, including Sambrook, J et al., (1989) Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory Press; Coligan, J. et al. (1994) Current Protocols in Immunology, Wiley & Sons, Incorporated; Enna, S.J. et al. (1991) Current Protocols in Pharmacology, Wiley & Sons, Bonifacino, J.S. et al. (1999) Current Protocols in Cell Biology, Wiley & Sons. All references mentioned herein are incorporated in their entirety.

EXAMPLES

[0113] $p210^{Bcr\text{-}Abl\text{-}T315I}$ is a theramutein of the p210Bcr-Abl protein ($p210^{Bcr\text{-}Abl}$) that is resistant to inhibition by imatinib mesylate (Gleevec, STI-571). The mutation at position 315 converts a threonine to an isoleucine residue and is one of several mutations that are observed among resistant or relapsed patients. This particular mutant, however, is the most resistant such theramutein yet identified.

[0114] A responsive phenotypic characteristic was determined for a Ba/F3 cell line engineered to overexpress the $p210^{Bcr\text{-}Abl\text{-}T315I}$ theramutein. The responsive phenotypic characteristic was determined relative to non-transformed Ba/F3 cells and Ba/F3 cells that express the $p210^{Bcr\text{-}Abl\text{-}wt}$ prototheramutein. The responsive phenotypic characteristic was the ability of the T315I mutants to grow to a higher cell saturation density under analogous culture conditions as compared to the control non-transformed Ba/F3 cell line, and to grow in

the absence of interleukin 3 (IL-3), which is required for maintenance of the control non-transformed Ba/F3 cell line.

[0115] The detection system utilized was a high speed cell imaging and counting system in which 3 μl sample volumes of cells were sequentially injected through a 5 μl optical microcell, digitally imaged and stored on computer, scanned, and then counted, all under a microcomputer-based control system. The system has the capacity to perform direct cell counts on samples from cultures as small as 500 μl and provides statistically significant total cell counts from culture samples containing as few as 12,500 cells. All of the figures displaying cell count and viability assays utilized this system for data acquisition and analysis. Simultaneously with the cell count performed, the system is also capable of determining overall cell viability by distinguishing counted, imaged cells that have excluded trypan blue (counted as "viable" cells) from cells which have taken up the trypan blue dye (counted as "non-viable" cells). Injection of trypan blue into the cell sample occurs immediately prior to the sample being sequentially injected into the microcell for simultaneous cell counting and imaging.

[0116] The system may be integrated into the workflow of high-throughput screening devices to provide a sensitive and precise cell counting and cell viability assay system that is more reliable and less prone to confounding effects of metabolic viability-based cellular assays such as XTT or Alamar blue.

[0117] Initially, approximately 113,000 compounds were screened at concentrations ranging from 10 to 20 μM to identify a subset that was capable of affecting growth of Ba/F3 cells (Ba/F3 T315I cells) overexpressing the $p210^{Bcr\text{-}Abl\text{-}T315I}$ theramutein by any means.

[0118] A total of approximately 11,760 compounds showed greater than 50% growth inhibition, which were thought to correspond to approximately 4500 distinct chemical classes. Retesting of these compounds with the same cell line yielded a database of compound responsiveness which was then sorted and rank ordered according to those compounds exhibiting the highest overall growth inhibition. From this rank ordered database, the highest scoring 130 compounds based upon the greatest degree of growth inhibition observed at the lowest concentrations that compounds were tested) were then rescreened in a defined cell-based assay system using Ba/F3 T315I as test cells and wild type Ba/F3 as control cells according to the methods of the present invention.. Compounds of interest were those that differentially inhibited growth of Ba/F3 cells expressing the

$p210^{Bcr\text{-}Abl\text{-}T315I}$ theramutein relative to non-transformed wild type Ba/F3 cells. Six compounds were identified that fulfilled the desired criteria, and some of these compounds were analyzed in further detail using the Ba/F3 $p210^{Bcr\text{-}Abl\text{-}wt}$ cells line (Ba/F3 P210 cells) as well. One compound was unavailable for further testing due to lack of availability of additional material from the chemical supplier. The remaining five compounds were independently evaluated in additional cell-based assays using the aforementioned cell lines as well as in a cell-free purified protein kinase assay using human recombinantly produced 120 Kd kinase domain fragments isolated from both wild type P210 Bcr-Abl as well as P210 T315I mutant kinase domain.

[0119] Compound C-2 inhibited $p210^{Bcr\text{-}Abl\text{-}T315I}$ 120 Kd activity as measured by inhibition of autophosphorylation activity, as shown in Figure 3. Other compounds identified in analogous manner will be the subject of other patent applications.

[0120] Taken together, the teachings and the results described herein provide conclusive proof that the system is capable of identifying inhibitors or activators of the selected theramutein, and the skilled investigator will immediately recognize that such a system may be easily applied to any other theramutein with only obvious, minor modifications.

[0121] Representative examples of the cell-based assay results demonstrating selective inhibition of growth of the Ba/F3 T315I cell line relative to the wild type non-transformed Ba/F3 cells are shown in Figure 1 . The compound inhibited growth and reduced the viability of cells expressing the T315I theramutein at concentrations under which the growth and viability of the wild type Ba/F3 non-transformed cells (not expressing either $p210^{Bcr\text{-}Abl\text{-}wt}$ or $p210^{Bcr\text{-}Abl\text{-}T315I}$) were relatively unaffected, whereas cells expressing both the prototheramutein as well as the theramutein were substantially inhibited.

[0122] In summary, the methods presented herein provide a fundamental advance in the form of a generalizable approach for creating or identifying modulators of any given theramutein The results demonstrate conclusively the power of the method to identify critically needed compounds to overcome a specific type of acquired drug resistance that is uniformly fatal in certain patient populations and is presently untreatable. Furthermore, it is evident to one of skill in this art that the techniques and methods described herein may, using obvious modifications, be straighforwardly generalized to any potential theramutein of clinical significance.

[0123] All references to any publication, patent, or other citation are hereby incorporated by reference.

Patent References

Senechal, K., Halpern, J., Sawyers, C.L. **The CRKL Adaptor Protein Transforms Fibroblasts and Functions in Transformation by the BCR-ABL Onocogene.** The Journal of Biological Chemistry, Volume 271 (September 20, 1996) Pages 23255-23261

Daley, G. Q., Van Etten, R. A., Baltimore, D. **Induction of Chronic Myelogenous Leukemia in Mice by the P210 $^{bre/abl}$ Gene of the Philadelphia Chromosome.** Science, Vol 247 (February 16, 1990) Pages 824-830

Druker, B. J., M.D., Talpaz, M., M.D., Resta, D.J., R.N., Peng, B., Ph.D., Buchdunger, E., Ph.D., Ford, J.M., M.D., Lydon, N. B., Ph.D., Kantarjian, H., M.D., Capdeville, R., M.D., Ohno-Jones, S., B.S., Sawyers, C. L., M.D. **Efficacy and Safety of a Specific Inhibitor of the BCR-ABL Tyrosine Kinase in Chronic Myeloid Leukemia.** The New England Journal of Medicine, Volume 344 (April 5, 2001) Pages 1031-1037

Druker, B.J., M.D., Sawyers, C.L., M.D., Kantarjian, H., M.D., Resta, D. J., R.N., Reese, S.F., M.D., Ford, J.M., M.D., Capdeville, R., M.D., Talpaz, M., M.D. **Activity of a Specific Inhibitor of the BCR-ABL Tyrosine Kinase in the Blast Crisis of Chronic Myeloid Leukemia and Acute Lymphoblastic Leukemia with the Philadelphia Chromosome.** The New England Journal of Medicine,Volume 344 (April 5, 2001) Pages 1038-1042

Faderl, S., M.D., Talpaz, M., M.D., Estrov, Z., M.D., O'Brien, S., M.D., Kurzrock, R., M.D., Kantarjian, H. M., M.D. **The Biology of Chronic Myeloid Leukemia.** The New England Journal of Medicine, Volume 341 (July 15,1999) Pages 164-172

Sawyers,C.L. M.D. **Chronic Myeloid Leukemia.** The New England Journal of Medicine, Volume 340 (April 29,1999) Pages 1330-1340

Schindler, T., Bornmann,W., Pellicena, P., Miller, W. T., Clarkson, B., Kuriyan, J. **Structural Mechanism for STI-571 Inhibition of Abelson Tyrosine Kinase.** Science, Volume 289 (September 15, 2000) Pages 1938-1942

Corbin, A. S., Buchdunger, E., Pascal, F., Druker, B. J. **Analysis of the Structural Basis of Specificity of Inhibition of the Abl Kinase by STI571**. The Journal of Biological Chemistry, Volume 277 (August 30, 2002) Pages 32214-32219

La Rosee, P., Corbin, A. S., Stoffregen, E. P., Deininger, M.W., Druker, B. J. **Activity of the Bcr-Abl Kinase Inhibitor PD180970 Against Clinically Relevant Bcr-Abl Isoforms That Cause Resistance to Imatinib Mesylate (Gleevec, STI-571).** Cancer Research, Volume 62 (December 15, 2002) Pages 7149-7153

Weisberg, E., Griffin, J. D. **Mechanism of Resistance to the ABL Tyrosine Kinase Inhibitor STI 571 in BCR/ABL-Transformed Hematopoietic Cell Lines.** Blood, Volume 95 (June 1, 2000) Pages 3498-3505

Le Coutre, P., Tassi, E., Varella-Garcia, M., Barni, R., Mologni, L., Cabrita, G., Marchesi, E., Supino, R., Gambacorti-Passerini, C.
Induction of Resistance to the Abelson Inhibitor STI571 in Human Leukemic Cells Through Gene Amplification. Blood, Volume 95 (March 1, 2000) Pages 1758-1766

Mahon, F. X., Deininger, M. W.N., Schultheis, B., Chabrol, J., Reiffers, J., Goldman, J.M., Melo, J.V. **Selection and Characterization of BCR-ABL Positive Cell Lines with Differential Sensitivity to the Tyrosine Kinase Inhibitor STI571: Diverse Mechanisms of Resistance.** Blood, Volume 96 (August 1, 2000) Pages 1070-1079

Allen, P.B., Wiedemann, L.M.
An Activating Mutation in the ATP Binding Site of the ABL Kinase Domain.
The Journal of Biological Chemistry, Volume 271 (August 9, 1996) Pages 19585-19591

Branford, S., Rudzki, Z., Walsh, S., Grigg, A., Arthur, C., Taylor, K., Hermann, R., Lynch, K.P., Hughes, T.P. **High Frequency of Point Mutations Clustered Within the Adenosine Triphosphate-Binding Region of BCR/ABL in Patients with Chronic Myeloid Leukemia or Ph-positive Acute Lymphoblastic Leukemia Who Develop Imatinib (STI571) Resistance.** Blood, Volume 99 (May 1, 2002) Pages 3472-3475

Druker, B. J., Tamura, S., Buchdunger, E., Ohno, S., Segal, G.M., Fanning, S., Zimmermann, J., Lydon, N.B.
Effects of a Selective Inhibitor of the Abl Tyrosine Kinase on the Growth of Bcr-Abl Positive Cells. Nature Medicine, Volume 2 (May 1996) Pages 561-566

Gambacorti-Passerini, C., Barni, R., Le Coutre, P., Zucchetti, M., Cabrita, G., Cleris, L., Rossi, F., Gianazza, E., Brueggen, J., Cozens, R., Pioltelli, P., Pogliani, E., Corneo, G., Formelli, F., D'Incalci, M. **Role of α1 Acid Glycoprotein in the *In Vivo* Resistance of Human BCR-ABL$^+$. Leukemic Cells to the Abl Inhibitor STI571.** Journal of the National Cancer Institute, Volume 92 (October 18, 2000) Pages 1641-1650

Gorre, M.E., Mohammed, M., Ellwood, K., Hsu, N., Paquette, R., Rao, P.N., Sawyers, C.L. **Clinical Resistance to STI- 571 Cancer Therapy Caused by BCR- ABL Gene Mutation or Amplification.** Science, Volume 293 (August 3, 2001) Pages 876-880

Hofmann, W. K., Jones, L.C., Lemp, N.A., DeVos, S., Gschaidmeier, H., Hoelzer, D., Ottmann, O. G., Koeffler, H. P. **Ph$^+$ Acute Lymphoblastic Leukemia Resistant to the Tyrosine Kinase Inhibitor STI571 has a Unique BCR-ABL Gene Mutation.** Blood, Volume 99 (March 1, 2002) Pages 1860-1862

Senechal, K., Heaney, C., Druker, B., Sawyers, C.L.
Structural Requirements for Function of the Crkl Adapter Protein in Fibroblasts and Hematopoietic Cells. Molecular and Cellular Biology, Volume 18 (September 1998) Pages 5082-5090

Barthe, C., Cony-Makhoul, P., Melo, J.V., Reiffers, J., Mahon, F,X.
Roots of Clinical Resistance to STI- 571 Cancer Therapy.
Science, Volume 293 (September 21, 2001) Page 2163a

Von Bubnoff, N., Schneller, F., Peschel, C., Duyster, J.

BCR-ABL Gene Mutations in Relation to Clinical Resistance of Philadelphia-Chromosome-Positive Leukaemia to STI571: A Prospective Study.
The Lancet, Volume 359 (February 9, 2002) Pages 487-491

Cunningham, B.C., De Vos, A.M., Mulkerrin, M.G., Ultsch, M, Wells, J.A.
Selecting Ligand Agonists and Antagonists.
U.S. Patent 5,506,107 (April 9,1996)

Cunningham, B.C., Wells, J.A., Clark, R. G., Olson, K., Fuh, G.G.
Method for Inhibiting Growth Hormone Action
U.S. Patent 6,004,931 (December 21,1999)

Wakai, T., Kanda, T., Hirota, S., Ohashi, A., Shirai, Y. Hatakeyama, K.
Late Resistance to Imatinib Therapy in a Metastatic Gastrointestinal Stromal Tumour is Associated With a Second KIT Mutation. British Journal of Cancer (April 20, 2004) Pages 1-3

Weigel, U., Meyer, M., Sebald, W. **Mutant Proteins of Human Interleukin 2. Renaturation Yield, Proliferative Activity and Receptor Binding.** European Journal of Biochemistry, Volume 180 (March 15, 1989) Pages 295-300.

Hou, Y.Y., Tan, Y.S., Sun, M.H., Wei, Y.K., Xu, J.F., Lu, S.H., A-Ke-Su, S.J., Zhou, Y.N., Gao, F., Zheng, A.H., Zhang, T.M., Hou, W.Z., Wang, J., Du, X., Zhu, X.Z.
C-kit Gene Mutation in Human Gastrointestinal Stromal Tumors. World Journal of Gastroenterology, Volume 10 (May 1, 2004) Pages 1310-1314

Noble, M.E.M., Endicott, J.A., Johnson, L.N. **Protein Kinase Inhibitors: Insights into Drug Design from Structure.** Science, Volume 303 (March 19, 2004) Pages 1800-1805

Lynch, T.J., M.D., Bell, D.W., Ph.D., Sordella, R., Ph.D., Gurubhagavatula, S., M.D., Okimoto, R.A., B.S., Brannigan, B.W., B.A., Harris, P.L., M.S., Haserlat, S.M., B.A., Supko, J.G., Ph.D., Haluska, F.G., M.D., Ph.D., Louis, D.N., M.D., Christiani, D.C., M.D., Settleman, J., Ph.D., Haber, D.A., M.D., Ph.D. **Activating Mutations in the Epidermal Growth Factor Receptor Underlying Responsiveness of Non-Small-Cell Lung Cancer to Gefitinib.** The New England Journal of Medicine, Volume 350 (May 20, 2004) Pages 2129-2139

Marx, J. **Why a New Cancer Drug Works Well, in Some Patients.** Science, Volume 304 (April 30, 2004) Pages 658-659

Paez, J.G., Jänne, P.A., Lee, J.C., Tracy, S., Greulich, H., Gabriel, S., Herman, P., Kaye, F.J., Lindeman, N., Boggon, T.J., Naoki, K., Sasaki, H., Fujii, Y., Eck, M.J., Sellers, W.R., Johnson, B.E., Meyerson, M. ***EGFR* Mutations in Lung Cancer: Correlation with Clinical Response to Gefitinib Therapy.** Sciencexpress (April 29, 2004) Pages 1-4

Leonard, G.D., Fojo, T., Bates, S.E. **The Role of ABC Transporters in Clinical Practice.** The Oncologist, Volume 8 (2003) Pages 411-424

Adcock, I.M., Lane, S.J. **Mechanisms of Steroid Action and Resistance in Inflammation.** Journal of Endocrinology, Volume 178 (2003) Pages 347-355

Loutfy, M.R., Walmsley, S.L. **Salvage Antiretroviral Therapy in HIV Infection.** Expert Opinion, Volume 3 (2002) Pages 81-90

Sambrook and Russell, **Molecular Cloning: A Laboratory Manual.** Cold Spring Harbor Laboratory Press, New York, 2001, Volumes 1-3

Foreman, J. C. and Johansen, T. **Textbook of Receptor Pharmacology** CRC Press, 2002; Boca Raton

11: Blood. 2004 Sep 30;
A cell-based screen for resistance of Bcr-Abl positive leukemia identifies the mutation pattern for PD166326, an alternative Abl kinase inhibitor.
Von Bubnoff N, Veach DR, Van Der Kuip H, Aulitzky WE, Sanger J, Seipel P, Bornmann WG, Peschel C, Clarkson B, Duyster J.

White, MF, Livingston, JM, Backer, Lauris, V, Dull, TJ, Ullrich A, Kahn, CR.
Mutation of the Insulin Receptor at Tyrosine 960 Inhibits Signal Transmission but Does Not Affect Its Tyrosine Kinase Activity.
Cell, Vol. 54, 641-649; 1988.

Leukemia. 2004 Oct 21
Histone deacetylase inhibitor NVP-LAQ824 has significant activity against myeloid leukemia cells in vitro and in vivo.
Weisberg E, Catley L, Kujawa J, Atadja P, Remiszewski S, Fuerst P, Cavazza C, Anderson K, Griffin JD.

3: Blood. 2004 Oct 15;104(8):2532-9. Epub 2004 Jul 15. Related Articles, Links
Inhibition of wild-type and mutant Bcr-Abl by AP23464, a potent ATP-based oncogenic protein kinase inhibitor: implications for CML.
O'Hare T, Pollock R, Stoffregen EP, Keats JA, Abdullah OM, Moseson EM, Rivera VM, Tang H, Metcalf CA 3rd, Bohacek RS, Wang Y, Sundaramoorthi R, Shakespeare WC, Dalgarno D, Clackson T, Sawyer TK, Deininger MW, Druker BJ.

5: Leukemia. 2004 Aug;18(8):1352-6. Related Articles, Links
Efficacy of dual-specific Bcr-Abl and Src-family kinase inhibitors in cells sensitive and resistant to imatinib mesylate.
Tipping AJ, Baluch S, Barnes DJ, Veach DR, Clarkson BM, Bornmann WG, Mahon FX, Goldman JM, Melo JV.

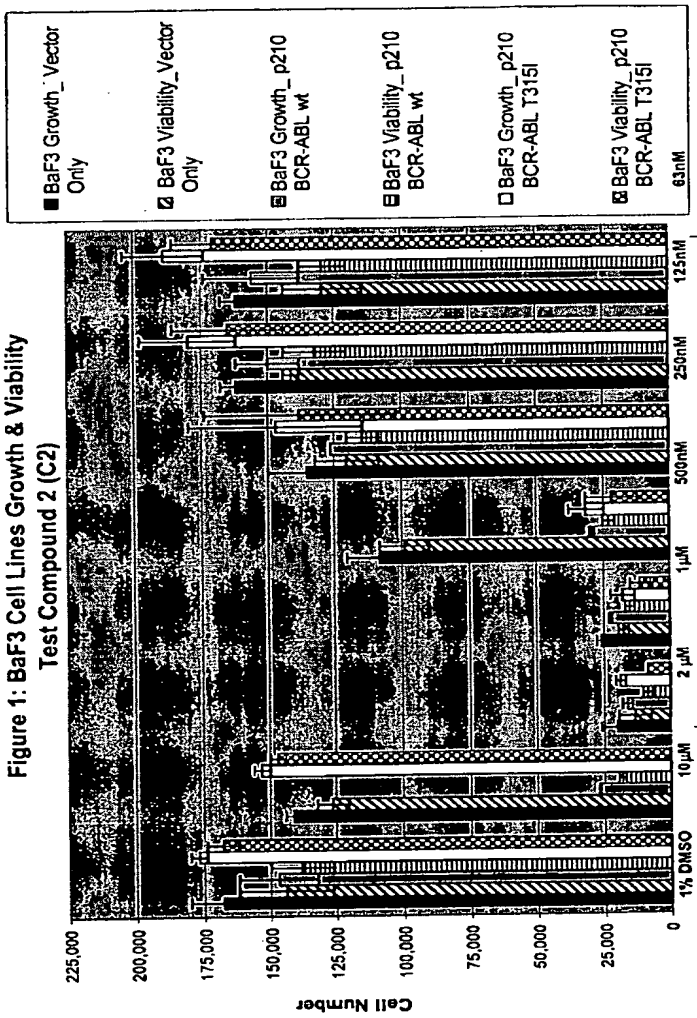


Fig. 1

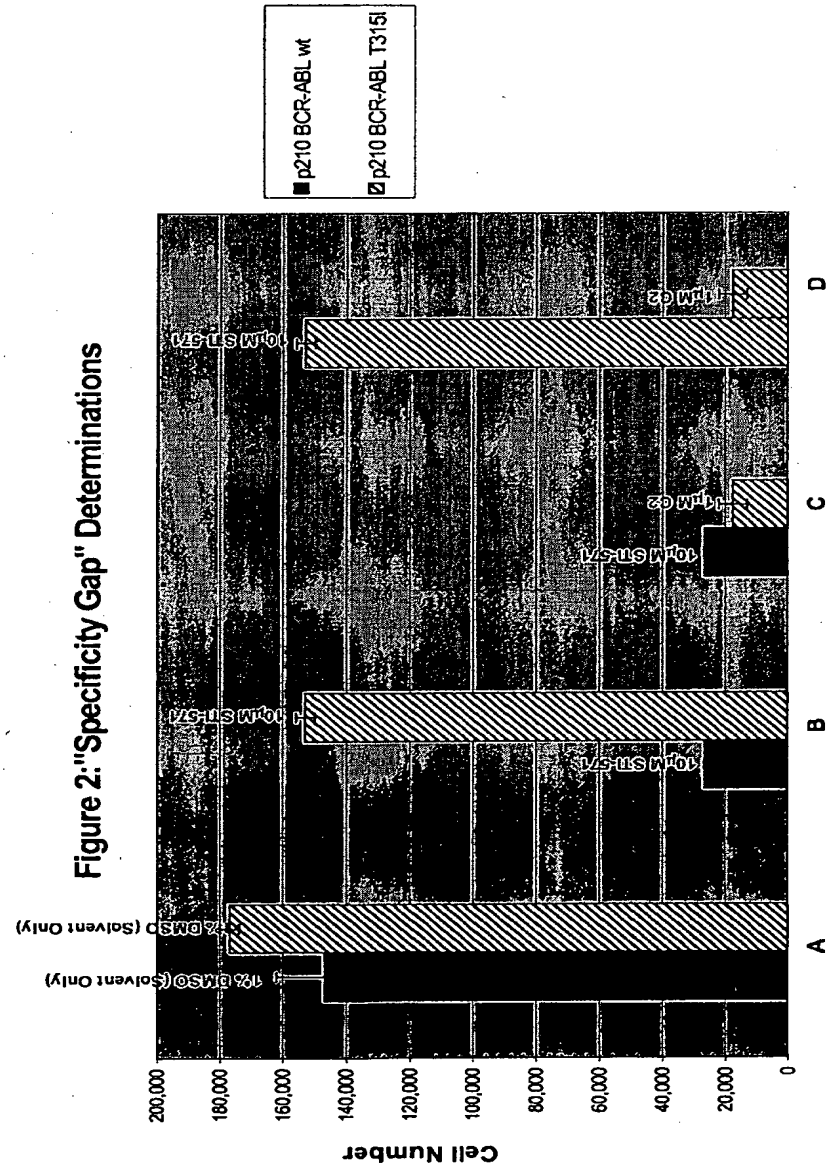
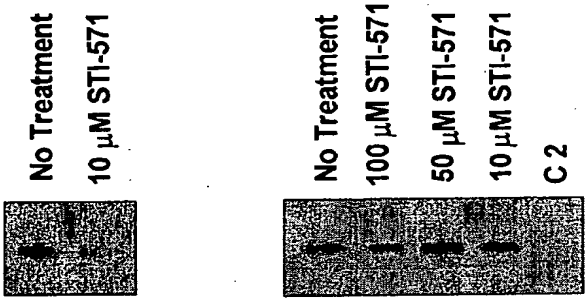


Fig. 2

Figure 3: Novel Inhibitor of the Abelson T315I Mutant Kinase



Relative Band Intensity*	100	43.8	100	100	100	100	35.3
% Inhibition	0	56.2	0	0	0	0	64.7

wt P210 T315I

*Determined by Digital Scanned Image Analysis